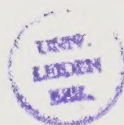


**α -Adrenoceptors
and Calcium Dependent
Contractions in
Isolated Cerebral and
Peripheral Arteries**

by
Tor Skärby

Lund 1984



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Abstract The postjunctional α -receptor in the cat middle cerebral artery (CMCA) was found to be mainly of α_2 -type whereas the α -receptor in cat mesenteric arteries (CMA) appeared to be unable to differentiate between antagonists that in other tissues act preferentially on α_1 - or α_2 -receptors. In human pial, temporal and omental (HOA) and cat lingual (CLA) arteries the α -receptor was predominantly of the α_1 -type. However, biphasic Schild plots in HOAs and CLAs suggested that also α_2 -receptors may contribute to the contractile response in these vessels. Pre- and postjunctional α_2 -receptors cannot be influenced separately by these drugs. The α_1 -receptor-mediated contractions in the rat middle cerebral artery (RMCA) seemed to depend on both Ca^{2+} influx and intracellular Ca^{2+} release whereas α_2 -receptor-mediated contractions in the CMCA were found to almost entirely rely on Ca^{2+} influx. The Ca^{2+} channels activated by α_2 -receptor stimulation in the CMCA were highly sensitive to nifedipine and had similar characteristics as the potential operated channels. In the RMCA the Ca^{2+} entry pathway was partly resistant to nifedipine. Nifedipine, verapamil and diltiazem were equally potent in relaxing the CMCA and the CMA contracted by K^+ . However, nimodipine was more potent in the CMCA suggesting that this drug had a certain selectivity for cerebral arteries. The intracellular store from which NA can release Ca^{2+} appeared to be larger in mesenteric than in cerebral arteries. Although the tonic component of the NA-induced contraction was dependent on Ca^{2+} influx, nifedipine reduced this component more in cerebral than in mesenteric arteries, suggesting that the Ca^{2+} entry pathway was more resistant to nifedipine in mesenteric than in cerebral arteries. The nifedipine resistant part of the tonic component appeared to be related to the extent to which NA releases intracellular Ca^{2+} , i.e. the greater the intracellular Ca^{2+} release, the greater the nifedipine resistance of the tonic component.	
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TOR SKÄRBY

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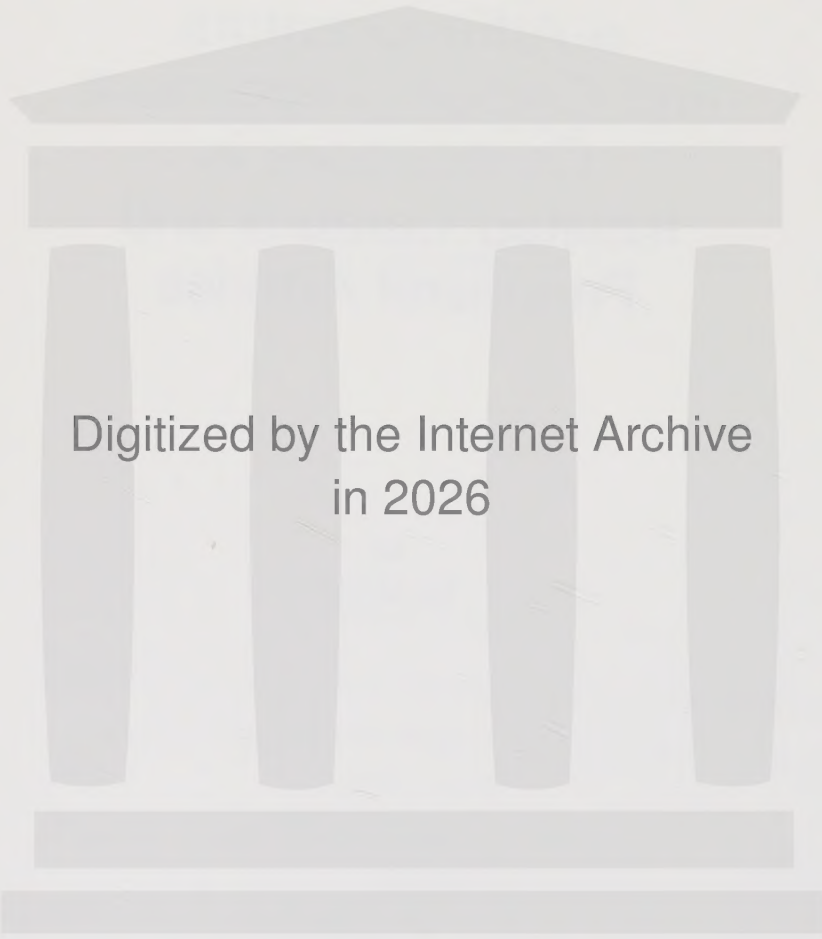
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Bestigningens möda är ingen måttstock med vilken
man mäter bergets höjd.

Nietzsche

The present thesis is based on the following articles, which will be referred to in the text by their Roman numerals.

- I. T.V.C. Skärby, K.-E. Andersson & L. Edvinsson: Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand*, 1983, 117:63-73.
- II. T. Skärby, & K.-E. Andersson: Contraction-mediating α -adrenoceptors in isolated human omental, temporal, and pial arteries. Submitted for publication.
- III. T. Skärby: Pharmacological properties of prejunctional α -adrenoceptors in isolated feline middle cerebral arteries; comparison with the postjunctional α -adrenoceptors. *Acta Physiol Scand*, 1984. Accepted for publication.
- IV. K.-E. Andersson, L. Edvinsson, E.T. MacKenzie, T. Skärby, & A.R. Young: Influence of extracellular calcium and calcium antagonists on contractions induced by potassium and prostaglandin $F_{2\alpha}$ in isolated cerebral and mesenteric arteries of the cat. *Br J Pharmacol*. 1983, 79:135-140.
- V. T. Skärby, E.D. Högestätt & K.-E. Andersson: Influence of extracellular calcium and nifedipine on α_1 - and α_2 -adrenoceptor mediated contractile responses in isolated rat and cat cerebral and mesenteric arteries. Submitted for publication.

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Abbreviations: α -receptors - α -adrenoceptors; β -receptors - β -adrenoceptors; K_B - dissociation constant; NA - noradrenaline; CLA - cat lingual artery; CMA - cat mesenteric artery; CMCA - cat middle cerebral artery; HOA - human omental artery; HPA - human pial artery; HTA - human temporal artery; POC - potential operated Ca^{2+} channel; RMA - rat mesenteric artery; RMCA - rat middle cerebral artery; ROC - receptor operated Ca^{2+} -channel; VSM - vascular smooth muscle.



INTRODUCTION

Transmitter in the sympathetic nervous system

Based on experiments in several animal species, Oliver & Schäfer (1895) demonstrated that extracts from the adrenals caused arterial constriction and elevation of blood pressure. The active substance isolated from these extracts, called adrenaline (Takamine 1901), was suggested to be equivalent to the chemical agent released from sympathetic nerves during electrical stimulation (Elliott 1905). Several years later von Euler demonstrated that the predominant catecholamine in peripheral tissues and adrenergic nerves was noradrenaline (NA) and not, as previously believed, adrenaline (von Euler 1946, 1948, 1951). Pearth (1949) was the first to conclusively show that noradrenaline was the main catecholamine released during stimulation of sympathetic nerves.

Adrenoceptor classification

Langley (1905) forwarded the hypothesis that adrenaline and other agents generated their effects by interacting with "receptive substances" on the effector organ. He also considered "that a cell may make motor or inhibitory receptive substances or both, and that the effect of a nervous impulse depends upon the proportion of the two kinds of receptive substance which is affected by the impulse" (Langley 1905). Studying a large number of smooth muscle preparations, Dale (1906) found that adrenaline could induce two disparate types of effect; in certain tissues the substance induced contractions, whereas other preparations responded with relaxation. After ergot pretreatment the contractile effects were either strongly reduced, or turned into a relaxation, further supporting the theory that adrenaline interacted with different types of receptive substance on the effector organ.

In contrast to the functional classification of adrenaline receptors into excitatory (motor) and inhibitory, Ahlquist (1948) suggested that "adrenotropic" receptors should be subclassified pharmacologically. Examining the effects of six sympathomimetic amines, Ahlquist (1948) found one relative order of potency in some tissues, whereas the order of potency was entirely different

in other preparations. He suggested that "the variations in activity are presumably due to actual differences in the receptors involved". Furthermore, these receptors could not "be classified simply as excitatory or inhibitory since each kind of receptor may have either action depending upon where it is found". Ahlquist therefore suggested that the receptors mediating e.g. vasoconstriction, excitation of the uterus and ureters, contraction of the nictitating membrane, dilatation of the pupil and inhibition of the gut should be called α -receptors. β -receptors he termed those which mediated vasodilation, inhibition of the uterus, and myocardial stimulation. Using a similar approach, Lands (1967) subclassified the β -receptors into β_1 - and β_2 -types.

α -receptor subclassification

Brown and Gillespie (1957) discovered that phenoxybenzamine, an α -receptor antagonist, increased the efflux of ^3H from cat spleen preincubated in ^3H -noradrenaline. Phenoxybenzamine also augmented the NA release in cultured ganglia where no postjunctional sites were present (Kopin et al 1973). Furthermore, the enhancing effect by α -receptor antagonists on the release of NA was not correlated to the type of receptor (α or β) in the effector organ (see Langer 1977). Based on these and other observations, it was suggested that α -receptors, in addition to being situated post-junctionally, also could be located on the adrenergic nerve endings (prejunctional α -receptors; for review see Starke 1981a). When stimulated, either by released NA or by blood borne catecholamines, these receptors mediate a negative feed-back, inhibiting subsequent release of transmitter.

Langer (1974) found phenoxybenzamine to be 30 times less potent on the pre- than on the postjunctional α -receptors, indicating that these receptors were not identical. He therefore suggested that the prejunctional α -receptor should be called α_2 and the postjunctional α -receptor should be referred to as α_1 . However, further studies localized α -receptors with the same pharmacological characteristics as the prejunctional α_2 -receptors on structures other than noradrenergic terminal axons (see Starke 1981b).

Berthelsen & Pettinger (1977) suggested that α -receptors should be classified according to their functional characteristics, rather than their anatomical localization. Wikberg (1978, 1979) and Starke & Langer (1979) generalized however the system for classification and proposed that α -receptors should be subclassified solely according to their pharmacological characteristics, disregarding the anatomical localization or the functional response they produced. This mode of classification is nowadays generally applied.

Contraction-mediating α_1 - and α_2 -receptors

An early indication for the presence of postjunctional α_2 -receptors mediating contraction in vascular smooth muscle was reported in the study by Moulds & Jauernig (1977). These authors found that NA-induced contractions in visceral arteries were competitively blocked by the α_1 -receptor selective antagonist prazosin whereas the contractile responses evoked by NA in digital arteries were relatively unaffected by the antagonist. Further studies (Stevens & Moulds 1981) using subtype selective agonists and the α_2 -receptor selective antagonist yohimbine suggested that both α_1 - and α_2 -receptors may be present postjunctionally in these vessels (see Flavahan & McGrath 1984). Examining the contraction-mediating α -receptors in isolated dog arteries and veins, De Mey & Vanhoutte (1981) obtained results indicating the presence of both α_1 - and α_2 -receptors in saphenous and femoral veins, whereas the contraction mediating α -receptors in the femoral and splenic arteries were mainly of α_1 -type.

The pressor effect induced by NA in pithed rats and anaesthetized cats was found to be relatively resistant to blockade by prazosin (Bentley et al. 1977). Further in vivo studies on rat (Kobinger & Pichler 1980, Timmermans & van Zwieten 1980, van Meel et al. 1983), rabbit (Madjar et al. 1980, van Zwieten et al. 1982), cat (Gardiner & Peters 1982, van Zwieten et al. 1982), dog (Constantine et al. 1980, Langer et al. 1980, Gardiner & Peters 1982) and man (Amann et al. 1980, van Brummelen et al. 1982, Elliott & Reid 1983) confirmed the presence of both α_1 - and α_2 -receptors postjunctionally in the cardiovascular system, as stimulation of either subtype could cause a blood

pressure increase or reduce the limb blood flow.

The studies of De Mey & Vanhoutte (1981), Ruffolo et al (1981) and Horn et al. (1982) suggest that the relative importance of postjunctional α_1 - and α_2 -receptors varies in different vascular regions and that either subtype may predominate. It might therefore be possible to selectively influence the circulation in different tissues with selective α_1 - and α_2 -receptor agonists and antagonists.

Pre- and postjunctional α_2 -receptors

Most studies which have examined the pharmacological characteristics of prejunctional α -receptors indicate that these receptors are of α_2 -type (cf. Langer 1981). If pre- and post-junctional α_2 -receptors have the same pharmacological characteristics, an α_2 -receptor antagonist administered in order to reduce α_2 -receptor mediated vasoconstriction would therefore also block prejunctional α_2 -receptors. Consequently, an increased outflow of noradrenaline from sympathetic nerves, causing unwanted effects, could be expected. Although the present nomenclature implies that pre- and postjunctional α_2 -receptors do not differ in their sensitivity to α -receptor agonists and antagonists, it has been suggested that the α -receptor antagonist HEAT (BE 2254) can discriminate between these types of α_2 -receptor (Hicks 1981).

Ca²⁺ and contraction of VSM

It is now generally accepted that the intracellular concentration of free Ca²⁺ is the final regulator of VSM contraction (Ebashi 1980). Receptor stimulation produces contraction through a release of Ca²⁺ from structures within the cell (e.g. sarcoplasmic reticulum and mitochondria) and/or an influx of Ca²⁺ from the extracellular medium (Steinsland et al. 1973, Bolton 1979, Deth & van Breemen 1974, Cauvin et al. 1982a, Awad et al. 1983). High K⁺-solutions are, on the other hand, generally believed to induce contraction by depolarization and a subsequent Ca²⁺-influx from the extracellular medium (Bolton 1979). Bolton (1979) suggested the presence of two different channels allowing Ca²⁺ from the extracellular medium into the smooth muscle cells to activate the contractile proteins: the potential operated channel (POC), opened by cell membrane depolarization and the

receptor operated channel (ROC) activated by agonists such as NA. In most tissues the contractions induced by depolarization were found to be considerably more sensitive to blockade by organic Ca^{2+} entry blockers (Ca^{2+} -antagonists) such as nifedipine, verapamil and diltiazem, than were those induced by agonists.

It has been shown that Ca^{2+} -antagonists predominantly block the stimulation-evoked influx of Ca^{2+} from the extracellular medium, and appear to have little or no effect on intracellular Ca^{2+} -release in therapeutically relevant concentrations (Cauvin et al. 1983). Moreover the influx of Ca^{2+} via ROCs is in most tissues considerably more resistant to blockade by Ca^{2+} antagonists than the influx via POCs (Cauvin et al. 1983). However, recent studies indicate that ROCs, in certain preparations, may be equally or even more sensitive to Ca^{2+} antagonists than the POCs (Walus et al. 1981, Cauvin et al. 1982b, Bevan 1983, Godfraind & Miller 1983).

α_1 - and α_2 -receptor mediated contractions and Ca^{2+} mechanisms.

Several authors have suggested that α_1 - and α_2 -receptors are linked to different pathways which increase the myoplasmic Ca^{2+} concentration. Studying isolated canine arteries and veins, De Mey & Vanhoutte (1981) obtained results indicating that vasoconstriction mediated by α_1 -receptors was more sensitive to verapamil than α_2 -receptor mediated contractions. This made them conclude that α_1 -receptor stimulation promotes mainly Ca^{2+} influx whereas activation of α_2 -receptors predominantly induces intracellular Ca^{2+} release. However, based on studies of pressor responses in the pithed rat, van Meel et al. (1981) suggested the opposite: α_1 -receptor activation liberated Ca^{2+} from intracellular stores whereas α_2 -receptor-mediated contractions were dependent on Ca^{2+} influx from the extracellular medium. Subsequent studies in pithed rats (Cavero et al. 1983), ganglion-blocked rabbits and pithed cats (see Van Zwieten et al. 1982), in perfused rat and dog hindlimb (Van Meel et al. 1983, Llenas & Massingham 1983) and in isolated dog saphenous vein (Cavero et al. 1983) correlate well with the results by van Meel et al. (1981).

Localization of postjunctional α -receptors

Based on studies in the isolated perfused dog hindlimb Langer et al. (1980) suggested that α_1 -receptors were concentrated at the neuroeffector junction and mediated the responses to neuronally released NA, whereas α_2 -receptors, prevailing "extra-synaptically", mediated the responses to circulating catecholamines. This agrees well with an earlier suggestion by Ariëns & Simonis (1976) who proposed that besides α -receptors for the transmitter NA, which they called α_T , there ought to be an α -receptor mediating the effects of the hormone adrenaline, α_H . The hypothesis by Langer et al. (1980) was further supported by a review of Ariëns & Simonis (1983). A study by Gardiner & Peters (1982) suggested, however, that neuronally released, as well as exogenous NA activated both α_1 - and α_2 -receptors in dog and cat hindlimb. Postjunctional α_2 -receptors have also been suggested to respond to neuronally released NA in the rat portal vein (Docherty & Starke 1981), canine trachea (Barnes et al., 1983) and in the pithed rabbit preparation (McGrath 1982). These findings do not support the general validity of the hypothesis put forward by Ariëns & Simonis (1976, 1983) and Langer and coworkers (1980, 1982) regarding a differential localization of α_1 - and α_2 -receptors.

Other types of contraction-mediating α -receptors ?

Most studies performed on α -receptor subtypes conform well with a classification into α_1 - and α_2 -receptors. However, a few investigators have suggested the existence of other types of contraction-mediating NA-receptors. The γ -receptor (Hirst & Neild 1980, Neild & Zelcer 1982) was proposed to be located on the smooth muscle of arteries at sites close to the nerves, and to respond to neuronally released NA. The receptor was activated only by high concentrations of NA ($> 10^{-4}$ M) causing a depolarization of the smooth muscle cells. The depolarization mediated by this receptor was not antagonized by α -receptor antagonists such as phentolamine. The existence of such a receptor is presently under debate (Bevan 1984, Neild & Hirst 1984).

Ruffolo et al. (1980, 1981, 1982) examined the affinity of yohimbine and clonidine for α -receptors in a number of different tissues. Based on the potencies of these drugs they suggested the

existence of three types of α -receptors with high, low, and intermediate affinities, respectively, for these drugs.

AIMS OF THE STUDY

The main objectives of the present investigation were:

1. To study regional variations in the pharmacological characteristics of contraction mediating α -receptors in cerebral and peripheral arteries from cat and man (I,II,III).
2. To compare pre- and postjunctional α_2 -receptors (I,III).
3. To compare Ca^{2+} -mechanisms involved in contractions mediated by α_1 - and α_2 -receptors (V).
4. To elucidate differences between cerebral and mesenteric arteries in the excitation-contraction coupling process (IV, V).

MATERIAL AND METHODS

Tension measurements

Cerebral and mesenteric arteries, with diameters ranging between 0.1 and 0.5 mm, were excised from cats and rats. From some cats lingual arteries of similar dimension were also removed. Human omental, temporal and pial arteries with a diameter of 1/2 - 2 mm were removed during surgery. The specimens were immediately put into cold buffer solution, and dissected free from adherent tissue. Ring segments (1 - 3 mm long) were thereafter suspended on two L-shaped metal holders immersed in tissue baths (see Fig 1). One of the holders was fixed to a movable unit allowing fine adjustments of the passive tension. The other holder was attached to a Grass FT 03C transducer and a Grass polygraph for continuous registration of the "isometric" tension of the vessel. The bath fluid was kept at 37° C and was continuously bubbled with 95 % O₂ and 5 % CO₂, giving a pH of approximately 7.4. During an equilibration period of 1/2 - 2 hours the arteries were frequently stretched until a steady passive tension of 2 - 8 mN was reached. The length of the equilibration period, and the amplitude of the passive tension given, varied between different types of artery. The passive tension applied was lower and the equilibration period was shorter in small (cerebral) than in large (temporal) arteries.

In most types of artery, contractile responses remained relatively reproducible for several hours. However, tachyphylaxis was frequently encountered in human omental and pial arteries. In the omental arteries this was compensated for by examining five or six segments from the same omental artery in parallel. Each segment was exposed to only one cumulative addition of agonist, and two of the segments were always used as controls receiving no drug but NA. A similar approach as that used in omental arteries was not possible in the human pial arteries, as the variation between different segments from the same artery was pronounced and the supply of tissue was scarce. Results from pial vessels essentially devoid of tachyphylaxis suggested that prazosin had non-competitive effects in this preparation, since the antagonist reduced the maximum response to NA. It would therefore be difficult to analyze the data according to Arunlakshana & Schild

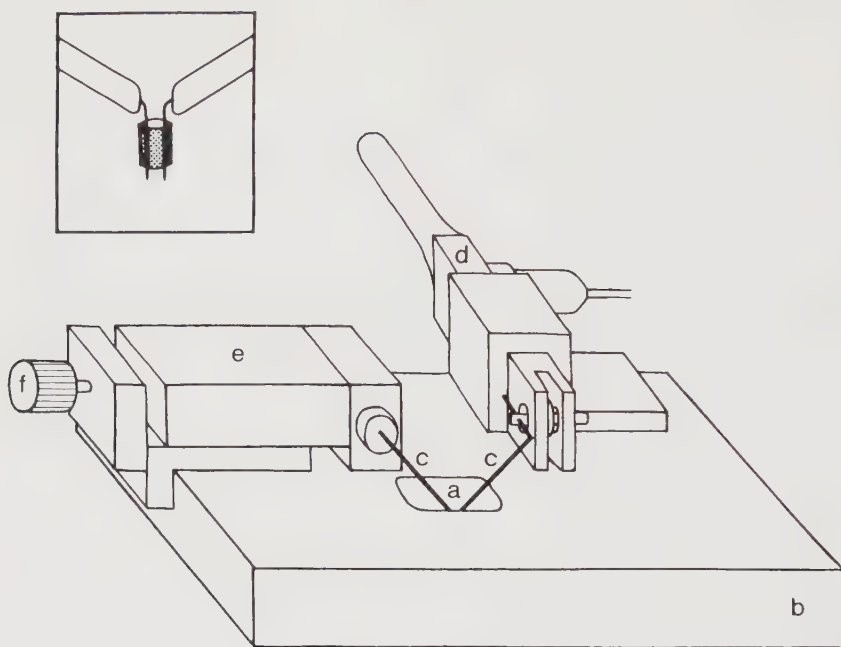


Fig 1. Schematic drawing of the myograph used for tension measurements in isolated vessels: a) muscle bath; b) perspex block. Two small stainless steel wires (diameter 0.2 mm) were attached to the distal ends of the specimen holders (c; see inset). One of these holders was connected to a Grass Instrument FT 03C force-displacement transducer (d). The opposite specimen holder was fixed to a displacement unit (e), which could be manipulated by a micrometer screw (f), and used to adjust the resting tension in the preparation. A gas mixture was conveyed through a small tubing, to the bottom of the muscle bath. Temperature-controlled water was continuously circulating around the muscle bath inside the perspex block.

(1959). As single applications of NA (10^{-5} M) appeared to give more reproducible responses, the potency of rauwolscine and prazosin in inhibiting single contractions induced by NA were therefore examined instead.

Release experiments

Feline cerebral arteries were incubated in buffer solution containing ^3H -noradrenaline for 45 min. After thorough washing the specimens were placed between two metal nets in perspex chambers (see Fig 2) and superfused with buffer solution. Temperature was kept at 37°C and the perfusion fluid was bubbled with 95 % O_2 and 5 % CO_2 giving a pH of approximately 7.4. Collection of the superfusate was started 150 min after onset of superfusion, and the preparations were stimulated electrically during at most 6 periods, each having a duration of 150 sec. The interval between each stimulation start was 21 min. The ^3H -content of each sample was determined by liquid scintillation counting, and was expressed as percentage of remaining tissue radioactivity (fractional release).

Calculations

E_{max} denotes the maximum effect by a drug and the concentration of drug at which 50 % of maximum effect occurs is defined as the EC_{50} -value. pEC_{50} (or pD_2) i.e., the negative logarithm of the EC_{50} -value was determined graphically from each curve. PA_2 -values, i.e., the negative logarithm of the antagonist concentration which displaces an agonist concentration-response (cr) curve towards higher concentrations with a factor of two were, unless otherwise stated, calculated according to Arunlakshana & Schild (1959) (Schild plot). The ratio of EC_{50} -values in the absence and presence of antagonist is designated the concentration ratio (CR).

The regressions in the Schild plots were based on linear least squares fit and the resulting values are given with the 95 % confidence intervals. Other values are given as mean $\pm$ SEM where n refers to the number of vessels examined. Statistical significance was determined by Student's t -test for paired or unpaired data. Apart from the effects of nifedipine on NA-induced contractions, which were compared by using analysis of variance, a probability level lower than 0.05 was accepted as significant.

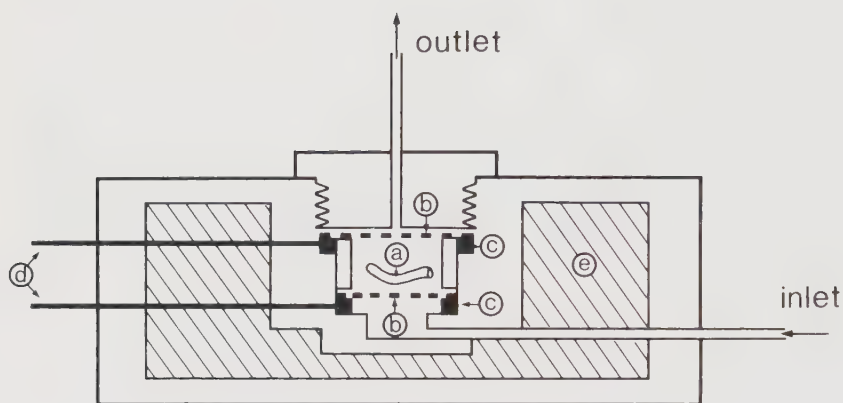


Fig 2 Schematic drawing of the perfusion chamber used in the release experiments: a) tissue; b) metal nets fixed to metal rings (c) connected to the stimulation electrodes (d); e) circulating, temperature controlled water.

In the Student's t-tests subsequent to the analysis of variance, differences were only accepted as significant when $p < 0.01$.

RESULTS

Postjunctional α -receptors in cat arteries (I, III)

Cerebral arteries

The initial contraction elicited by NA in the cat middle cerebral artery (CMCA) was usually small. However, the response to NA (10^{-5} M) increased after each subsequent exposure to the drug and reproducible contractions were generally not obtained until five or six contractions had been elicited (Skärby; unpublished observations). All agonists examined caused concentration-dependent contractions of the CMCA and the order of potency was: clonidine $\geq$ oxymetazoline $>$ NA $>$ phenylephrine.

The CMCA displayed a high sensitivity to yohimbine as demonstrated by a shift of the NA cr-curve by low concentrations of the drug (10^{-8} M). Prazosin did not appear to cause a parallel shift of the NA cr-curve. However, both antagonists caused significant reductions of the $E_{\max}$. The order of potency of antagonists to inhibit single contractions induced by NA 10^{-5} M was: rauwolscine $>$ HEAT (BE 2254) $>$ prazosin.

Peripheral arteries

Clonidine was devoid of contractile effect in cat mesenteric (CMA) and lingual (CLA) arteries. The relative order of potency of the other agonists was: oxymetazoline $>$ NA $>$ phenylephrine $>$ methoxamine. Neither prazosin, yohimbine nor rauwolscine caused any appreciable reduction of the maximum effect in the NA cr-curves in any of the concentrations used. In the CMA these antagonists caused concentration-dependent shifts of the agonist cr-curves, and the slopes of the regression lines in the corresponding Schild plots were close to unity (see Table 1).

Figure 3 shows the effects of rauwolscine, prazosin and phentolamine on the NA cr-curve in the CLA, investigated in presence of corticosterone 4×10^{-5} M, cocaine 10^{-5} M and propranolol 10^{-6} M (Skärby; unpublished observations).

This treatment increased significantly ($p < 0.001$) the pEC_{50} -value of the control NA cr-curve from 5.59 ± 0.04 ($n=9$) (in presence of 10^{-6} M cocaine and 10^{-7} M propranolol) to 6.06 ± 0.04 ($n=9$).

Table 1. Data on the Schild regressions illustrating the effects of α -receptor antagonists in cat peripheral arteries.

Artery	Antagonist	Agonist	z	Slope of regression line	Correlation coefficient	pA_2 -value
Lingual	Prazosin	NA	22	0.92 (0.80 - 1.05)	0.96	8.76 (8.55 - 9.04)
"-	Phentolamine	NA	24	0.92 (0.78 - 1.05)	0.95	7.74 (7.56 - 7.99)
"-	Rauwolscine	NA	60	0.79 (0.73 - 0.86)	0.96	8.01 (7.90 - 8.14)
Mesenteric	Prazosin	NA	29	0.93 (0.82 - 1.04)	0.96	8.70 (8.50 - 8.94)
"-	Yohimbine	NA	23	0.95 (0.77 - 1.12)	0.93	7.34 (7.16 - 7.56)
"-	Rauwolscine	NA	18	0.95 (0.81 - 1.08)	0.96	7.35 (7.20 - 7.54)
"-	"-	Phenyl- ephrine	23	0.88 (0.71 - 1.05)	0.92	7.39 (7.19 - 7.68)

Values within brackets represent the 95 % confidence intervals, and z denotes the number of points in each regression line. Experiments on mesenteric arteries were performed in presence of 10^{-6} M cocaine and 10^{-7} M propranolol, whereas the effects of drugs on lingual arteries were investigated in presence of 10^{-5} M cocaine, 10^{-6} M propranolol and 4×10^{-5} M corticosterone.

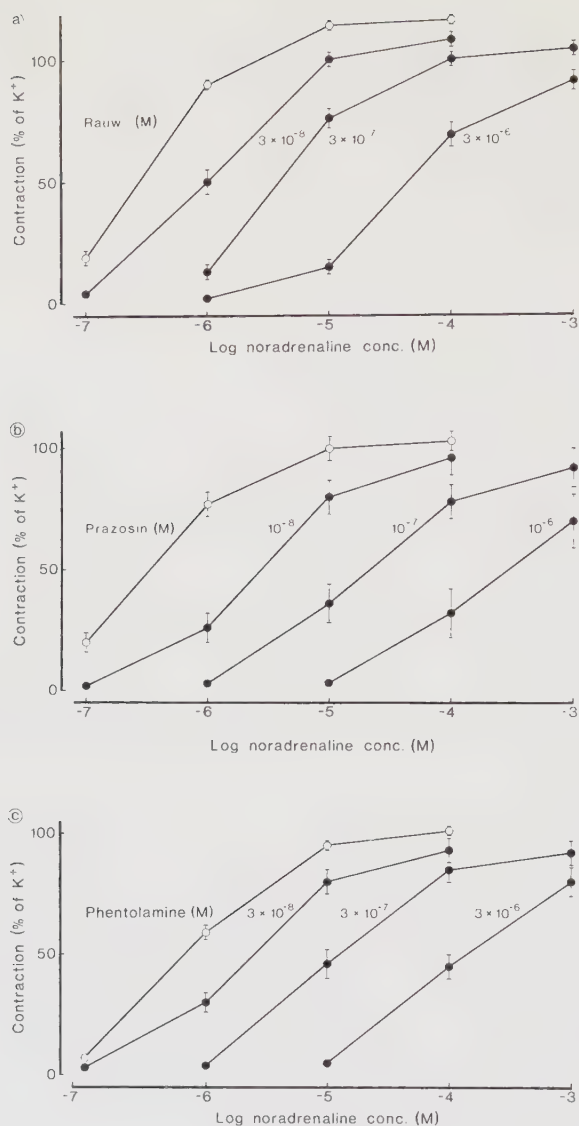


Fig 3 Concentration-response curves obtained by cumulative addition of NA to cat lingual arteries in absence (○) and presence (●) of a) rauwolscine (Rauw; n=11-12), b) prazosin (n=7-8) and c) phentolamine (n=8). Each value is expressed as a percentage of the K⁺-induced contraction. The buffer solution contained 10⁻⁶ M propranolol, 10⁻⁵ M cocaine and 4 × 10⁻⁵ M corticosterone. For clarity, the effects of rauwolscine are only shown in the concentrations 3 × 10⁻⁸, 3 × 10⁻⁷ and 3 × 10⁻⁶ M.

The Schild plot illustrating the antagonistic properties of rauwolscine in presence of these concentrations of corticosterone, cocaine and propranolol, appeared to be biphasic (Fig 4a). A regression line calculated for the effects of the concentrations 3×10^{-9} - 3×10^{-7} M had a slope of 0.66, which was significantly ($p < 0.001$) lower than unity. A regression line fitting the effects of 3×10^{-7} - 3×10^{-6} M of the drug had a slope of 1.04. If the entire plot was regarded as linear, and a single regression line was fitted to all the data points, the slope was 0.79 which differed significantly ($p < 0.001$) from unity. In contrast to the effects of rauwolscine, the effects of prazosin and phentolamine on the NA cr-curve, as illustrated in Schild plots appeared to fit well with single straight regression lines, having slopes close to unity (Fig 4b, see Table 1).

Postjunctional α -receptors in human arteries (II)

Cerebral arteries

The human pial arteries (HPA) showed a pronounced inter- and intra-individual variation in their response to NA, and in contrast to temporal (HTA) and omental arteries (HOA) most exhibited spontaneous activity. Clonidine was without contractile effects, whereas oxymetazoline was more, and phenylephrine less potent than NA. Prazosin 10^{-8} M effectively reduced the $E_{\max}$ of the NA cr-curves, whereas yohimbine 10^{-7} M was essentially devoid of effects on these NA-induced responses. Prazosin was considerably more potent than rauwolscine in inhibiting contractions elicited by single concentrations of NA.

Peripheral arteries

The agonists NA, phenylephrine and oxymetazoline caused concentration-dependent contractile responses in HTAs and HOAs, while clonidine exhibited essentially no contractile effects. The order of potency was: oxymetazoline > NA > phenylephrine. Both prazosin and rauwolscine caused rightward displacements of the NA cr-curve without appreciably affecting the $E_{\max}$. The regression lines in the Schild plots, illustrating the effects of prazosin (Fig 5), had slopes close to unity. The pA_2 -values were 9.21 and 9.45 in the HTAs and HOAs respectively. Rauwolscine in

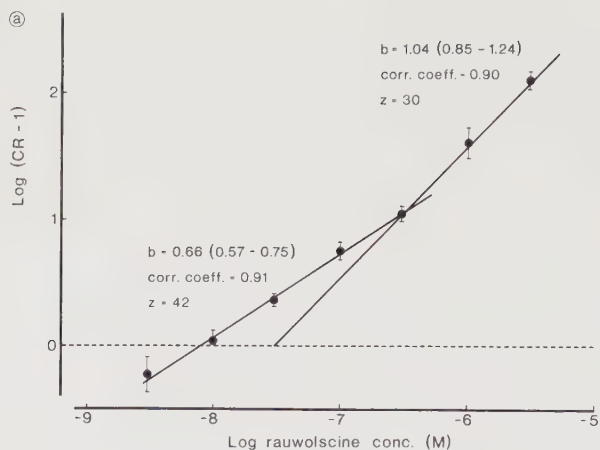


Fig 4 a Schild plot illustrating the effects of rauwolscine in cat lingual arteries ($n=6-12$), using NA as agonist: b) slope factor; z) number of points in the regression. The buffer solution contained 10^{-6} M propranolol, 10^{-5} M cocaine and 4×10^{-5} M corticosterone.

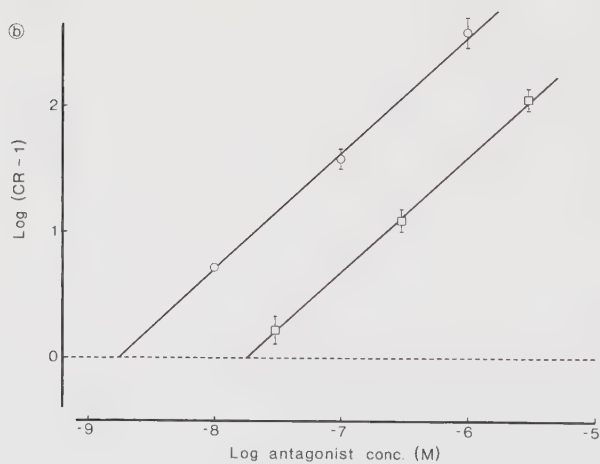


Fig 4 b Schild plots showing the effects of prazosin ($n=7-8$; ○) and phentolamine ($n=8$; □). For further details see legends to Fig 4 a.

concentrations below 3×10^{-6} M appeared to be unable to shift the NA cr-curve in the HTAs. The pA_2 -value estimated according to van Rossum (1963), was 6.15. In the HOAs 3×10^{-8} - 10^{-5} M of the antagonist caused significant displacements of the cr-curve. The Schild plot illustrating these effects had a biphasic appearance (see Fig 5). Additional experiments performed in the presence of cocaine 3×10^{-5} M, corticosterone 4×10^{-5} M and propranolol 3×10^{-7} M did not shift the control NA cr-curve leftwards or abolish the biphasic appearance of the Schild plot. The regression line drawn in the concentration interval 10^{-8} - 10^{-7} M, had a slope of 0.23, which differed significantly from unity. A regression line calculated in the concentration interval 10^{-6} - 10^{-5} M had, however, a slope of 0.99. If a single regression line was drawn for the effects of all the concentrations 3×10^{-8} - 10^{-5} M a slope of 0.67 was obtained, which differed significantly from unity.

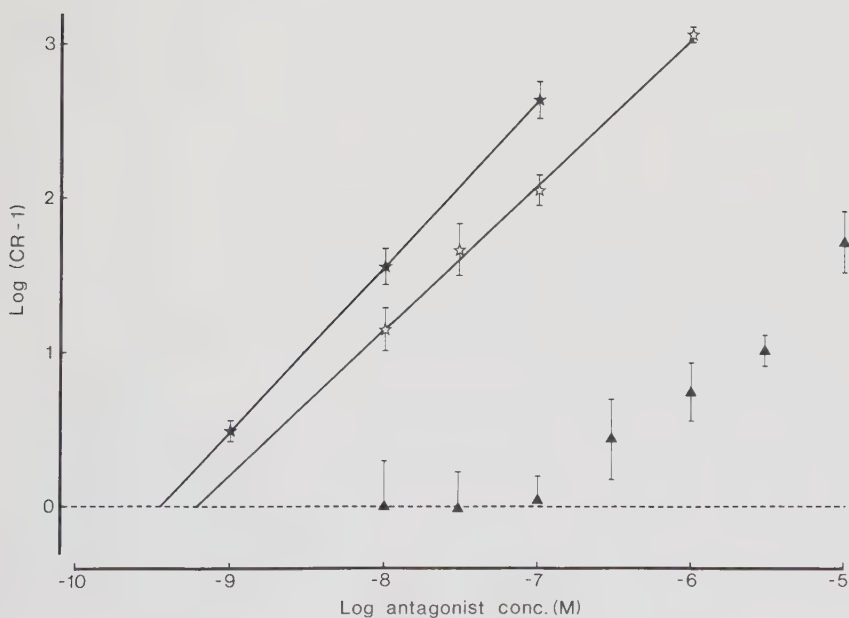


Fig 5 Schild plots illustrating the interaction between NA and prazosin in human omental (★) and temporal (☆) arteries; effects of rauwolscine in omental arteries (▲).

Prejunctional α -receptors in cat cerebral arteries (III)

In the CMCA preincubated with ^3H -NA, the outflow of ^3H evoked by electrical stimulation was increased by α -receptor antagonists and reduced by α -receptor agonists. The relative order of potency for agonists was: clonidine > oxymetazoline >> phenylephrine. The antagonists augmented release with the following order of potency: rauwolscine > HEAT (BE 2254) > prazosin. Figure 6 illustrates the effects of these antagonists on electrically evoked ^3H release and NA induced contractions in cat cerebral arteries.

Effects of Ca^{2+} antagonists on K^{+} -induced contractions in VSM (IV, V).

K^{+} -induced contractions in cat and rat cerebral and cat mesenteric arteries remained stable for more than 30 min. Nimodipine, nifedipine, verapamil and diltiazem all effectively relaxed K^{+} -induced contractions in the CMCA and CMA (Fig 7). The maximum relaxation produced by the different Ca^{2+} antagonists in the two types of artery varied between 90 and 98 %. The relative order of potency of the different drugs in the CMCA was: nimodipine > nifedipine > verapamil > diltiazem. Apart from nimodipine being of similar potency as nifedipine, the relative order of potency of Ca^{2+} antagonists was the same in the CMA. Nimodipine was approximately four times more potent in the CMCA than in the CMA, whereas the other drugs failed to discriminate between the two vascular beds. Treatment of the CMCA and CMA in Ca^{2+} -free medium almost abolished the K^{+} -induced contractions in the CMCA and CMA. Cumulative reapplication of Ca^{2+} caused immediate and concentration-dependent contractions, which could be inhibited by both nimodipine and nifedipine. In the rat middle cerebral artery (RMCA), nifedipine relaxed K^{+} -induced contractions with almost identical potency and maximum effect as in the CMCA.

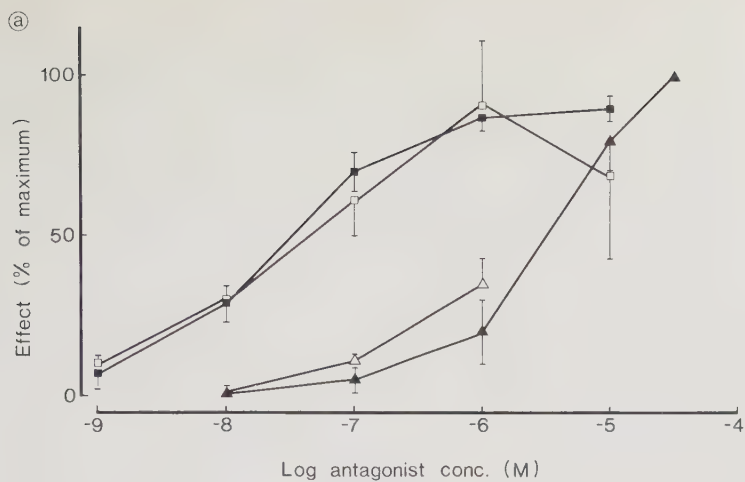


Fig 6 a Effects of rauwolscine (■) and prazosin (▲) on the contractions induced by 10^{-5} M NA (filled symbols) and the effects of these antagonists on the ^3H -release evoked by electrical stimulation (open symbols) in cat cerebral arteries. Both effects are expressed as a percentage of the maximum response caused by HEAT with respective experimental procedure.(See also study III).

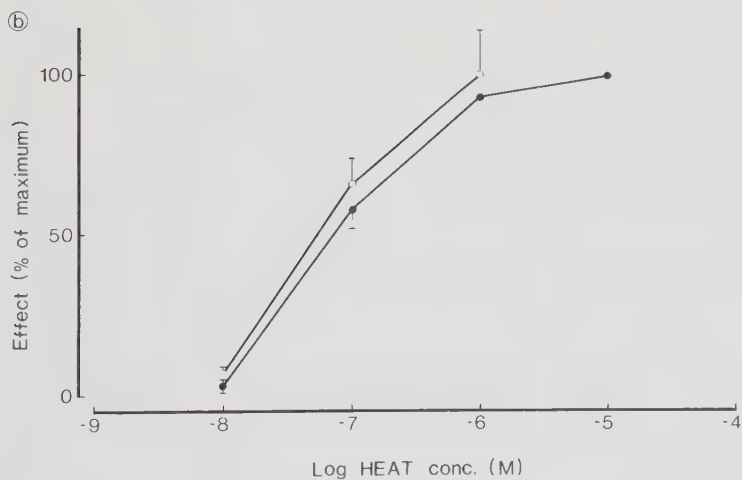


Fig 6 b Effects of HEAT in the cat middle cerebral artery. For explanations, see legends to Fig 5 a.

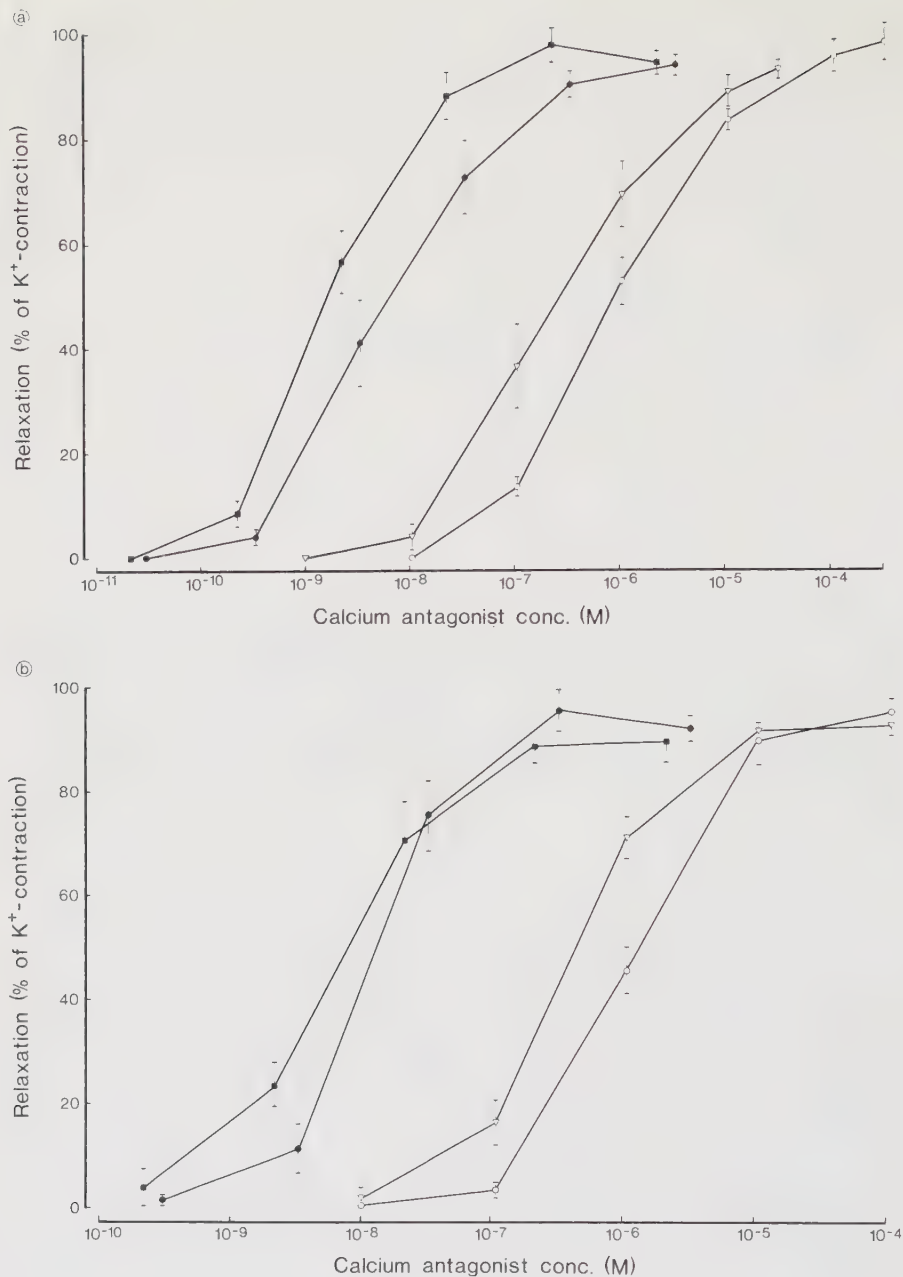


Fig 7 Concentration-relaxation curves for nimodipine (■), nifedipine (●) verapamil (▽) and diltiazem (○) in K⁺-contracted feline a) middle cerebral and b) mesenteric arteries.

Influence of Ca^{2+} removal and nifedipine on NA-induced contractions in VSM (V).

NA usually induced an initial rapid tension increase followed by a tonic response in the RMCA, RMA (rat mesenteric artery), and CMA. However, in the CMCA, the NA-induced contractions developed considerably slower than in the other types of vessel.

The tonic response to NA was substantially reduced by Ca^{2+} deprivation in all the different types of vessel studied. The amplitude of the maximum contractile response to 10 μM NA after 10 min in Ca^{2+} -free medium decreased in the following order between the various vessel segments: $\text{CMA} \approx \text{RMA} > \text{RMCA} \gg \text{CMCA}$. In the CMCA the NA-induced contractions were virtually abolished after this treatment. Excluding the CMCA, in which the responses to K^+ and NA were equally reduced by a 10 min preincubation period in Ca^{2+} -free medium, the K^+ -induced contraction was always more reduced than was the response to NA.

In contrast to the NA-induced contractions in the mesenteric arteries from the two species, the contractions evoked by NA in the cerebral arteries were relatively sensitive to nifedipine. Furthermore, in the CMCA these contractions were more sensitive to the Ca^{2+} antagonist than were those in the RMCA. Interestingly, nifedipine was approximately equipotent in inhibiting NA-induced contractions and in relaxing contractions evoked by K^+ in the CMCA. However, in the RMCA the contractile response to K^+ was slightly more sensitive to nifedipine than was the NA-induced contractions. The arteries could be ranked in the following order with respect to their ability to contract upon NA exposure in presence of nifedipine: $\text{RMA} \approx \text{CMA} \gg \text{RMCA} \gg \text{CMCA}$. A close correlation ($r=0.99$, $p < 0.01$) was found between the degree to which 3 μM nifedipine inhibited the tonic component of the NA-induced contraction, and the maximum amplitude of the NA-induced contraction after 10 min in Ca^{2+} -free medium (Fig 8).

In separate experiments it was observed that contractions induced by NA in nifedipine exposed arteries were unaltered (RMCA and RMA) or only slightly reduced (CMCA and CMA) by prior K^+ -depolarization.

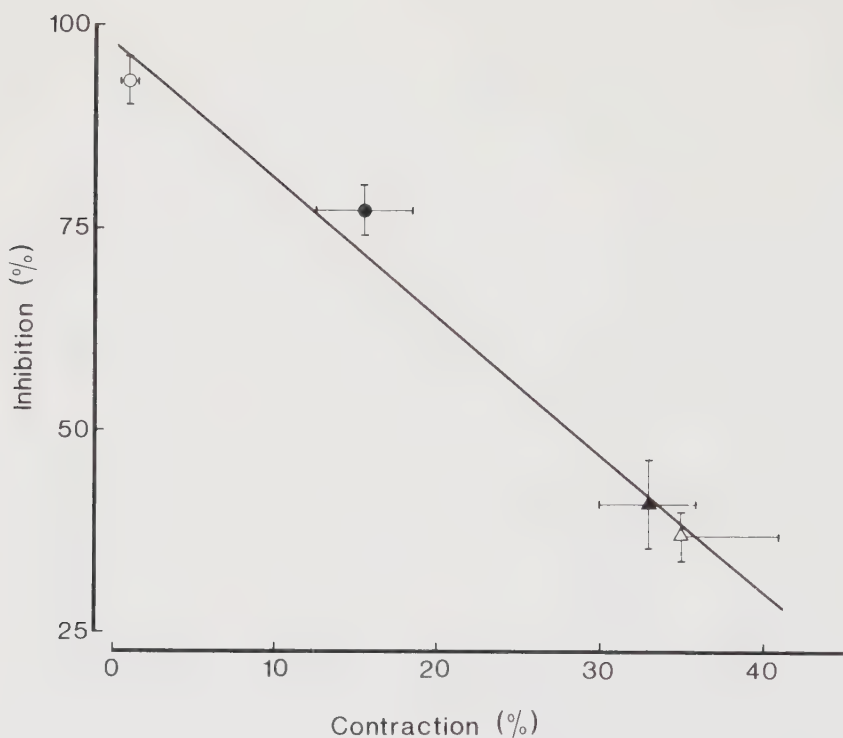


Fig 8 Linear correlation between the mean inhibition (ordinate) produced by nifedipine of the tonic component of the contractile response to 10 μ M NA, and the mean amplitude of the contraction (abscissa) elicited by NA after 10 min in Ca^{2+} free medium, as revealed by regression analysis. Cat middle cerebral artery (O); cat mesenteric artery (Δ); rat middle cerebral artery ($\bullet$); rat mesenteric artery ($\blacktriangle$). Each mean value was based on 5-9 experiments. For further explanations see study V.

The effects of nifedipine were also examined on contractions induced by re-application of Ca^{2+} to Ca^{2+} -depleted vessels exposed to 10 μM NA. In the RMCA, RMA, and CMA a considerable contraction could still be evoked after pretreatment with nifedipine. The Ca^{2+} -antagonists delayed, however, the contractile response to Ca^{2+} and made the tension increase less rapidly. In the CMCA, nifedipine almost completely abolished the Ca^{2+} -induced contraction.

Postjunctional α -receptors in cerebral arteries

The order of potency for agonists to produce contractile responses in the CMCA corresponds with that obtained on prejunctional α -receptors in rabbit pulmonary artery (Starke et al., 1975) and has been suggested to be representative for α_2 -receptors (see Langer 1980, Starke & Docherty 1980, Langer & Shepperson 1981, Starke 1981). Clonidine was 550, and NA 36 times more potent than phenylephrine. Furthermore, rauwolscine was approximately 100 times more potent than prazosin in inhibiting single contractions induced by 10^{-5} M NA (III) and yohimbine was considerably more potent than prazosin in shifting the NA cr-curve (I). Each of these observations indicate that the α -receptor mediating contraction in the CMCA is predominantly of α_2 -type.

Studying the effects of subtype selective α -receptor agonists and antagonists on the isolated perfused CMCA, Medgett & Langer (1983) obtained similar results confirming these findings. Recent in vivo studies in cat indicate that administration of yohimbine, but not prazosin, could shift the autoregulatory curve towards lower levels of arterial pressure (MacKenzie et al. 1983). This further substantiates the findings obtained in vitro, and indicates that the predominant α -receptor mediating cerebrovascular contractions in vivo is of α_2 -type.

In isolated human pial arteries (HPAs) prazosin appeared to be considerably more potent than yohimbine in affecting the NA cr-curves. However, low concentrations of prazosin seemed to cause a conspicuous reduction of $E_{\max}$. As the effects could be washed out and the response to NA restored, these findings suggest that prazosin had non-competitive, but not irreversible, effects in the HPAs. Such effects were also observed in cat cerebral arteries with both yohimbine and prazosin. Similar findings have previously been obtained in cerebral arteries from man, monkey (Toda 1983) and dog (Sakakibara et al. 1982, Toda 1983). In these vessels stimulation of β -receptors did not seem to be an explanation for the reduction in $E_{\max}$ caused by prazosin or yohimbine (Toda 1983). Although yohimbine has been suggested to have Ca^{2+} -antagonistic properties (Godfraind et al 1983), these

effects were obtained in concentrations considerably greater (30 μ M) than those used in Study I. Furthermore, Toda (1983) observed that neither prazosin 10^{-8} M nor yohimbine 10^{-8} M reduced K^{+} -induced contractions. Also contractions induced by 5-HT in brain arteries from several species were reduced in a non-competitive manner by antagonists, which appeared to interact competitively with 5-HT receptors in peripheral arteries (Edvinsson et al 1978, Lamar & Edvinsson 1980, Forster & Whalley 1982). The explanation for the non-competitive behaviour in cerebral vessels by antagonists which cause a competitive interaction in most peripheral arteries, is obscure.

Prazosin appeared to be substantially more potent than rauwolscine in inhibiting single contractions elicited by 10^{-5} M NA in the HPAs. Although the high potency of prazosin to a certain extent could be explained by a non-competitive effect, rauwolscine was considerably less potent in inhibiting the NA-induced response in these vessels than in cat cerebral arteries in which the contraction-mediating α -receptors appear to be mainly of α_2 -type (see above). The drug was also much less potent on postjunctional α -receptors in the HPAs than on prejunctional α_2 -receptors (see Weitzell et al., 1979, Docherty & Starke 1982, Study III). These results suggest that the α -receptor mediating contractions in the HPAs is predominantly of α_1 -type. This conclusion is further supported by the finding that phenylephrine but not clonidine elicited contractions in the HPAs. Examining the effects of yohimbine and prazosin on the NA curve elicited in human cerebral arteries obtained during autopsy, Toda (1983) came to the same conclusion regarding the postjunctional α -receptors in these vessels.

The contraction-mediating α -receptor in man, monkey (Toda 1983) and rat (Högestätt & Andersson 1984) cerebral arteries appears to be mainly of α_1 -type, whereas it seems to be predominantly of α_2 -type in the corresponding arteries from dog (Sakakibara et al. 1982, Toda 1983) and cat (see above). Thus the α -receptor subtype mediating contractions in cerebral arteries seems to vary considerably between different species.

Comparison of pre- and postjunctional α_2 -receptors in cat cerebral arteries

Electrically evoked ^3H -efflux from the CMCA preincubated with ^3H -NA was decreased by α -receptor agonists and enhanced by α -receptor antagonists. The order of potency of agonists and antagonists suggest that the α -receptors through which the modulation of release is mediated (prejunctional α -receptors) are predominantly of α_2 -type. This is in good agreement with results obtained in most other studies (see e.g. Sullivan & Drew 1980, Docherty & Starke 1982, Frankhuyzen & Mulder 1982, Reichenbacher et al. 1982).

Based on in vivo studies in the rat it was suggested that HEAT (BE 2254) was 185 times more potent on pre- than postjunctional α_2 -receptors in the cardiovascular system (Hicks 1981). This indicated that pre- and postjunctional α_2 -receptors may have different pharmacological characteristics; if so this finding might be of considerable clinical relevance. However, in contrast to the study by Hicks (1981), HEAT was found to lack the ability to discriminate between pre- and postjunctional α_2 -receptors in the CMCA. Furthermore, with the exception of clonidine, which appeared to have higher intrinsic activity pre- than post-junctionally, all the drugs studied had similar concentration-effect curves on pre- and postjunctional sites. Also the ratios of EC_{50} -values pre- and postjunctionally of rauwolscine, oxymetazoline, and clonidine, were all close to unity, suggesting that these drugs have similar potency on the two sites. These findings do not suggest that drugs interacting with α -receptors can discriminate between pre- and postjunctional α_2 -receptors; a conclusion which agrees well with recent results obtained in pithed rats (Paciorek & Shepperson 1983).

Postjunctional α -receptors in peripheral arteries

In contrast to clonidine, phenylephrine elicited contractions in all the peripheral arteries studied. The potency ratios of NA and phenylephrine obtained in these vessels correspond with that suggested to be representative for α_1 -receptors (see Starke 1981). Prazosin, rauwolscine and yohimbine displaced the NA curves towards higher concentrations in all vessels exposed to the drugs, without appreciable effects on E_{max} . This suggests a

competitive interaction of all the antagonists used.

In each vessel type studied, the antagonistic effect of prazosin, as illustrated in a Schild plot, resulted in a straight regression line. Each of these lines had a slope close to unity, indicating a simple competitive blocking effect of the drug. The pA_2 -values ranged between 8.70 and 9.45. These values correlate well with those obtained in a number of studies, in which prazosin has been suggested to interact with α_1 -receptors (see Table 2). Furthermore, prazosin was always considerably more potent than rauwolscine in shifting the NA cr-curve towards higher concentrations, which agrees well with the potency order of antagonists representative for α_1 -receptors. Thus, the hitherto discussed results obtained in peripheral arteries with agonists and antagonists suggest that the α -receptors mediating contractions in the CMAs and CLAs, HOAs and HTAs were predominantly of the α_1 -type.

The effects of α_2 -receptor antagonists varied however considerably between the different types of vessel studied. The Schild plots, illustrating the antagonistic effects of yohimbine and rauwolscine in the CMA, had slopes close to unity. The resulting pA_2 -values (7.34 and 7.35) should therefore be good estimations of the negative logarithm of the K_B -values (Arunlakshana & Schild 1959, Kenakin 1982). These pA_2 -values seem to agree better with those reflecting an interaction with α_2 -receptors than with those proposed to illustrate the potency of yohimbine and rauwolscine on α_1 -receptors (see Table 3 and 4). Yohimbine and rauwolscine are stereoisomers, the latter being the more selective of the two (Weitzell et al. 1979, Starke & Docherty 1980, McGrath 1982). This difference has been attributed mainly to a higher potency of yohimbine than rauwolscine on α_1 -receptors, whereas the two drugs were almost equally potent on the α_2 -receptors (see McGrath 1982). The findings that the Schild plots of yohimbine and rauwolscine had slopes which were superimposable and that the pA_2 -values of these drugs were identical, may therefore also indicate an interaction with α_2 -receptors.

Table 2. Affinity of prazosin for α_1 - or α_2 -receptors in different tissues.

pA_2	Tissue	Reference	
<u>α_1-receptors</u>			
7.9 ¹	Dog femoral artery	De Mey & Vanhoutte	1981
7.9 ¹	Dog splenic artery	" " "	1981
8.0 ¹	Human femoral artery	Glusa & Markwardt	1983
8.6 ¹	Rabbit aorta	Cavero et al.	1978
8.6 ²	" "	Awad et al.	1983
8.9 ¹	Rabbit uterus	Hoffman et al.	1981
9.0 ¹	Rat vas deferens	Minneman et al.	1983
9.2 ²	" " "	" " "	1983
9.3 ¹	Rat cerebral artery	Högestätt & Andersson	1984
9.3 ²	Rabbit uterus	Lavin et al.	1981
9.4 ²	Rabbit urethra	Andersson et al.	1984
9.7 ¹	Rat mesenteric artery	Högestätt & Andersson	1984
9.9 ¹	Human groin artery	Steen et al.	1984
9.9 ²	Guinea pig lung	Barnes et al.	1979
10.0 ²	Rat brain	Greengrass & Bremner	1979
<u>α_2-receptors</u>			
5.1 ²	Rabbit uterus	Lavin et al.	1981
5.1 ²	Rabbit urethra	Andersson et al.	1984
5.6 ²	Canine airway smooth muscle	Barnes et al.	1983
5.8 ²	Human platelets	Brodde et al.	1982

pA_2 -values were obtained from functional in vitro studies¹ in which the Schild plots had slopes not differing from unity. K_D -values, obtained in radioligand binding studies², were transformed to pA_2 -values ($-\log K_D = pA_2$).

Table 3. Affinity of yohimbine for α_1 - or α_2 -receptors in different tissues.

pA ₂	Tissue	Reference	
<u>α₁-receptors</u>			
5.5 ²	Guinea pig lung	Barnes et al.	1979
5.5 ²	Rabbit uterus	Lavin et al.	1981
6.0 ¹	Rat vas deferens	Ruffolo et al.	1981
6.0 ¹	Rabbit aorta	" " "	1982
6.0 ²	Rat brain	Greengrass & Bremner	1979
6.0 ²	Rabbit aorta	Awad et al.	1983
6.0 ²	Rat vas deferens	Minneman et al.	1983
6.1 ¹	Guinea pig aorta	Ruffolo et al.	1982
6.1 ¹	Rat vas deferens	Minneman et al.	1983
6.4 ¹	Human femoral artery	Glusa & Markwardt	1983
6.5 ¹	Dog femoral artery	De Mey & Vanhoutte	1981
6.5 ¹	Rabbit uterus	Hoffman et al.	1981
6.6 ¹	Rabbit pulmonary artery	Weitzell et al.	1979
6.6 ¹	Dog splenic artery	De Mey & Vanhoutte	1981
<u>α₂-receptors</u>			
7.3 ¹	Dog femoral vein	De Mey & Vanhoutte	1981
7.3 ¹	Human femoral vein	Glusa & Markwardt	1983
7.6 ¹	Rat tail artery	Weiss et al.	1983
7.8 ¹	Guinea pig ileum	Drew	1978
7.8 ¹	Adrenergic neuron in rat heart	Fuder et al.	1983
7.9 ²	Rabbit uterus	Lavin et al.	1981
8.4 ²	Human adipocytes	Tharp et al.	1981
8.5 ²	Human platelets	Brodde et al.	1982
8.6 ²	" "	Motulsku & Insel	1982
8.6 ²	Canine airway smooth muscle	Barnes et al.	1983
8.7 ¹	Dog basilar artery	Sakakibara et al.	1982

pA_2 -values were obtained from functional in vitro studies¹ in which the Schild plots had slopes not differing from unity. K_D -values, obtained in radioligand binding studies², were transformed to pA_2 -values ($-\log K_D = pA_2$).

Table 4. Affinity of rauwolscine for α_1 - or α_2 -receptors in different tissues.

pA ₂	Tissue	Reference	
<u>α₁-receptors</u>			
5.1 ²	Rabbit uterus	Lavin et al.	1981
5.1 ²	Rabbit urethra	Andersson et al.	1984
5.4 ¹	Rat cerebral artery	Högestätt & Andersson	1984
5.8 ¹	Human femoral artery	Glusa & Markwardt	1983
5.9 ¹	Rabbit pulmonary artery	Weitzell et al.	1979
<u>α₂-receptors</u>			
7.3 ²	Rabbit uterus	Lavin et al.	1981
7.5 ¹	Human femoral vein	Glusa & Markwardt	1983
8.1 ²	Rabbit urethra	Andersson et al.	1984
8.3 ²	Human platelets	Motulsky & Insel	1982
8.7 ²	" "	Brodde et al.	1982
9.0	Human groin vein	Steen et al.	1984

pA_2 -values were obtained from functional in vitro studies¹ in which the Schild plots had slopes not differing from unity. K_D -values, obtained in radioligand binding studies², were transformed to pA_2 -values ($-\log K_D = pA_2$).

The results obtained with prazosin suggest the presence of α_1 -receptors, whereas those obtained with yohimbine and rauwolscine would favour the occurrence of α_2 -receptors. If an agonist activates two types of receptor mediating the same type of response, and the antagonist blocks only one of these, the slope of the regression line in the Schild plot may be less than unity (Kenakin 1982). Despite this, and the fact that NA is an unselective α -receptor agonist, the slope of the Schild plots for prazosin, yohimbine and rauwolscine were all close to unity. Consequently, a strict interpretation of these findings would indicate that the antagonists all interfered with one type of receptor, which therefore may be regarded as "broader" in the sense that it appears to be unable to differentiate between antagonists that in other tissues preferentially interact with α_1 - or α_2 -receptors. Similarly, Ruffolo and coworkers found that in certain isolated smooth muscle preparations from rat, the affinity of yohimbine and clonidine was intermediate to that observed on α_1 - and α_2 -receptors. As the slopes of the Schild plots were not significantly different from unity, these authors suggested that the intermediate affinities of clonidine and yohimbine did not reflect a simultaneous occurrence of α_1 - and α_2 -receptors, but rather indicated that selective agonists and antagonists interacted with one type of receptor in these tissues (see Ruffolo et al. 1981).

In both CLAs (in presence of corticosterone 4×10^{-5} M, cocaine 10^{-5} M and propranolol 10^{-6} M) and HOAs, the Schild plot, illustrating the antagonistic properties of rauwolscine, seemed to be biphasic. It has been shown that the Schild plot could exhibit unusual characteristics when neuronal uptake and β -receptors are not sufficiently blocked (see Furchgott 1972, Kenakin 1982). However, in the HOAs corticosterone and high concentrations of cocaine and propranolol did not increase the pEC_{50} -value of the NA cr-curve or abolish the biphasic appearance of the plot. In the CLA, the slope obtained with phentolamine in presence of cocaine 10^{-5} M, corticosterone 4×10^{-5} M and propranolol 10^{-6} M was close to unity. These findings indicate that NA uptake mechanisms and β -receptors were adequately blocked during the present experiments in both CLAs and HOAs. Although rauwolscine has been suggested to have Ca^{2+} antagonistic properties in rat aorta

(Godfraind et al. 1983), preliminary experiments indicated that rauwolscine (10^{-5} M) did not relax K^{+} -contracted CLAs ($n=2$) and HOAs ($n=2$), suggesting the absence of such effects by the drug in these vessels.

As previously described, Kenakin (1982) suggested that the slope of the Schild plot may be lower than unity if the agonist activates two types of receptor and the antagonist blocks only one of these. Thus, the low slope obtained in the lower range of rauwolscine concentrations (3×10^{-9} - 10^{-7} M) may represent an interaction with a small population of α_2 -receptors. These concentrations of rauwolscine can indeed be regarded as interacting with α_2 -receptors with a relatively high degree of selectivity (Ferry et al. 1983, Andersson et al. 1984). As can be seen in Table 4, rauwolscine appears to yield pA_2 -values of 5.1 - 5.9 when interacting with α_1 -receptors. The effects of the higher rauwolscine concentrations (10^{-6} - 10^{-5} M) in the present Schild plot might therefore represent an interaction also with α_1 -receptors. Consequently, the antagonist may, in these concentrations behave as an unselective antagonist in the Schild plot giving a slope close to unity. Phentolamine which is an unselective α -receptor antagonist, gave a straight regression line with a slope of unity confirming that an unselective α -receptor blockade in the CLA actually yields what receptor theory predicts.

Schild plots and pA_2 -values were originally designed from homogenous receptor populations, and little is known of the appearance of the plot when more than one receptor population is involved. However, in a recent communication, Flavahan & McGrath (1984) suggested that yohimbine could produce a biphasic Schild plot, when both α_1 - and α_2 -receptors are present in the tissue. In good agreement with the present study, it was proposed that such a plot, when regarded as linear, would yield a slope of less than 1. However, even if a contribution of α_2 -receptors can not be excluded, the present results indicate that the contraction-mediating α -receptors in the CLAs and HOAs are mainly of α_1 -type.

Of the rauwolscine concentrations used in the HTAs only 3×10^{-6} M of the drug was able to significantly displace the NA cr-curve towards higher concentrations. The pA_2 -value based on the shift caused by 3×10^{-6} M rauwolscine was calculated according to van Rossum (1963). Although this value (6.15) can be regarded only as an estimation, it is considerably lower than pA_2 -values suggested to represent an interaction of this antagonists with α_2 -receptors. It correlates better with pA_2 -values proposed to describe the potency of rauwolscine on α_1 -receptors (see Table 4). The present results thus indicate that the α -receptor mediating contractions in the HTAs are mainly, if not exclusively, of the α_1 -type.

Although oxymetazoline has been suggested to have a preference for α_2 -receptors it seems consistently to be more potent than phenylephrine, irrespective of the α -receptor subtype stimulated by the drugs (see Wikberg 1979). The postjunctional α -receptor in both the rabbit pulmonary artery (Starke 1981, Starke & Docherty 1983) and rabbit aorta (Ruffolo et al. 1982, Cauvin et al. 1982a, Awad et al. 1983) has been found to be of α_1 -type and NA was 4 - 5 times more potent than phenylephrine in both types of vessel (see Wikberg 1979). In spite of these findings, oxymetazoline was 2.8 times more potent than phenylephrine in the pulmonary artery, whereas the corresponding potency difference was 25 in the aorta (see Wikberg 1979). The rat basilar artery has been suggested to lack contraction-mediating α -receptor (Neild & Zelcker 1982), which corresponds well with the finding that NA, phenylephrine and clonidine generally were unable to elicit contractions in this vessel (Högestätt & Andersson 1984). In spite of that, oxymetazoline was found to concentration-dependently contract this artery (Högestätt & Andersson 1984). Although these aberrant effects of oxymetazoline are interesting, it may be questioned if oxymetazoline can be regarded as a reliable tool in the present system for α -receptor subclassification.

α_1 - and α_2 -receptor mediated contractions and Ca^{2+} mechanisms

In the RMCA, NA usually induced an initial rapid response followed by a slow tonic component. However, in the CMCA, NA produced mainly a slow monophasic tension increase. As the α -receptors in the RMCA have been found to be mainly of the α_1 -type

(Högestätt & Andersson 1984) whereas the α_2 -type predominates in the CMCA (see above), these findings correlate to those obtained in pithed rats in which the pressor responses induced by α_1 -selective agonists developed considerably faster than those elicited by α_2 -receptor agonists (Timmermans & van Zwieten 1980, van Zwieten et al. 1982, Caverio et al. 1983). This difference in time course between α_1 - and α_2 -receptor mediated responses may reflect differences in the excitation-contraction coupling process associated with stimulation of the two types of receptor.

The fast response of the NA-induced contraction in several VSM preparations appears to be associated with mobilization of Ca^{2+} from intracellular stores whereas the tonic component is dependent on Ca^{2+} influx from the extracellular medium (Steinsland et al. 1973, Deth & van Breemen 1974, Bolton 1979). The results obtained with Ca^{2+} -free medium, in the present study, suggest that the Ca^{2+} utilized for contraction during the tonic component is derived mainly from the extracellular medium, irrespective of the α -receptor subtype stimulated by NA. The contractile response to K^+ in the RMCA and RMA was always more reduced by treatment in Ca^{2+} -free medium than that elicited by NA, suggesting that NA, in addition to mobilizing extracellular Ca^{2+} , also can liberate Ca^{2+} from intracellular stores in these arteries. These results do not support the view that α_1 -receptors are linked specifically to intracellular Ca^{2+} release (see Bou et al. 1983). Recent studies in other preparations have also indicated that α_1 -receptor stimulation in vascular smooth muscle induce both Ca^{2+} influx and intracellular Ca^{2+} release (Cauvin et al. 1982a, Awad et al. 1983, Manzini et al. 1983). In the CMCA, K^+ - and NA-induced contractions were equally suppressed and almost abolished after 10 min in Ca^{2+} free medium, suggesting that the contractile response to NA in this preparation rely almost entirely on Ca^{2+} influx. The failure of NA to release intracellularly stored Ca^{2+} in the CMCA might reflect a general lack of coupling between α_2 -receptors and intracellular Ca^{2+} release, as suggested by e.g., van Meel (1982).

NA- and K^+ -induced contractions in the CMCA were similarly sensitive to both nifedipine and Ca^{2+} removal. Furthermore, nifedipine almost abolished the contraction induced by Ca^{2+} in

arteries exposed to NA in Ca^{2+} free medium. These findings suggest that K^+ and NA largely utilize the same pathway to increase the myoplasmic Ca^{2+} concentration. Electrophysiological studies on isolated cat cerebral arteries have shown a close correlation between NA-induced contraction and membrane depolarization (Harder et al., 1981, Harder, 1983). It therefore seems possible that nifedipine inhibited the contractile response to NA in the CMCA by blocking POCs, activated by the NA-induced membrane depolarization.

Although the presence of POCs in the RMCA can not be excluded, three pieces of evidence indicate the existence of a nifedipine resistant Ca^{2+} entry pathway, possibly equivalent to ROC, in this vessel. First, nifedipine failed to abolish the tonic component of the contractile response to NA. Secondly, the nifedipine resistant part of the NA-induced contraction was present also after prior K^+ depolarization. Thirdly, the contraction induced by reintroduction of Ca^{2+} to the Ca^{2+} depleted RMCA was not abolished by nifedipine.

The differential effects of nifedipine on α_1 - and α_2 -receptor mediated contractions in the RMCA and CMA cannot be explained solely by a larger intracellular Ca^{2+} release in the RMCA. The difference is probably also attributable to the presence of a Ca^{2+} entry pathway resistant to nifedipine in the RMCA, but not in the CMCA.

Regional variations in the excitation-contraction coupling

As high K^+ solutions are generally believed to induce VSM contraction by depolarization and a subsequent influx of Ca^{2+} via POCs (Bolton 1979), the potency of different Ca^{2+} antagonists in relaxing K^+ -induced contractions may be used to compare these channels in different VSM preparations. Accordingly, the POCs in the CMCA and CMA appear to be similar, since K^+ -induced contractions in these arteries were equally affected by nifedipine, verapamil and diltiazem. However, nimodipine was 4 times more potent in relaxing the CMCA than the CMA, suggesting that this drug may have a certain selectivity for cerebral arteries. This is in contrast to the findings of Brandt et al. (1981) showing that nifedipine, but not nimodipine was more potent

in relaxing K^+ -contracted human pial than mesenteric arteries.

Several studies suggest that agonist-induced contractions in cerebrovascular smooth muscle can be more effectively blocked by Ca^{2+} antagonists than contractions in peripheral smooth muscle (Allen & Banghart 1979, Towart 1981, Towart et al. 1982, Bevan 1983). In the present study, nifedipine was considerably more potent in inhibiting NA-induced contractions in the RMCA and CMCA than in the RMA and CMA. Furthermore, the contractile response to NA was always reduced more by treatment in Ca^{2+} -free medium in the cerebral than in the mesenteric arteries. This suggests that the intracellular Ca^{2+} stores, releasable by NA, are larger in mesenteric than in cerebral arteries. The difference does not seem to be related to differences in vessel size as the rat arteries were always smaller than the cat arteries. Neither could the difference be explained by differences in the receptor type activated by NA, as the postjunctional α -receptors in both rat cerebral and mesenteric arteries are mainly of α_1 -type (Colucci et al. 1980, 1981, Högestätt & Andersson 1984).

The tonic component of the NA-induced contraction was always substantially reduced after short incubation periods in Ca^{2+} -free medium. This suggests that the contraction during this component is highly dependent on Ca^{2+} influx from the extracellular medium irrespective of the type of vessel studied. Despite this, nifedipine was considerably more potent in reducing the tonic component in cerebral than in mesenteric arteries. These findings suggest that the more pronounced effects by nifedipine on cerebral than mesenteric arteries may not solely be explained by differences in the intracellular stores releasable by NA, but also by differences in the nifedipine sensitivity of the pathway through which Ca^{2+} enters the cell.

Interestingly, the maximum inhibition of the tonic component produced by nifedipine appeared to be related to the amplitude of the contraction elicited by NA in Ca^{2+} -free medium in the different types of vessel studied. This suggests that the nifedipine resistant part of the tonic component somehow is related to the extent to which NA releases intracellular Ca^{2+} in that vessel, i.e., the greater the intracellular Ca^{2+} release

induced by NA, the greater the nifedipine resistance of the tonic response. Such a correlation would be anticipated if the nifedipine resistant part of the tonic component reflects a fast refilling of the NA sensitive intracellular Ca^{2+} store directly from the extracellular medium, and a subsequent release of Ca^{2+} into the myoplasm. Casteels & Droogmans (1981) have previously suggested that such a pathway, resistant to Ca^{2+} antagonists, may represent an alternative model of the ROC.

SUMMARY AND CONCLUSIONS

The contraction-mediating α -receptors in several vascular regions in cat and man, have been characterized by means of subtype selective agonists and antagonists. The α -receptors in the cat middle cerebral artery (CMCA) was found to be mainly of α_2 -type. A strict analysis of the Schild plots for the antagonists in cat mesenteric arteries (CMA), suggests the presence of a "broader" type of α -receptor unable to differentiate between antagonists that in other tissues act preferentially on α_1 - or α_2 -receptors. The results obtained in human pial, temporal and omental, and cat lingual arteries suggest that the contraction-mediating α -receptors in these vessels are predominantly of the α_1 -type. However, in cat lingual and human omental arteries the Schild plot for rauwolscine appeared to be biphasic, suggesting that also α_2 -receptors may contribute to the contractile response in these vessels.

Pre- and postjunctional α_2 -receptors were compared by examining the effects of selective agonists and antagonists on electrically evoked ^3H -NA release and on contractile responses in the CMCA. With the exception of clonidine which appeared to have higher intrinsic activity pre- than postjunctionally, none of the drugs examined (oxymetazoline, phenylephrine, rauwolscine, HEAT and prazosin) was able to discriminate between pre- and postjunctional α_2 -receptors.

Ca^{2+} mechanisms involved in contractions mediated by α_1 - and α_2 -receptors were compared in the CMCA and RMCA (rat middle cerebral artery). Contractions mediated by α_1 -receptors in the RMCA seem to depend on both Ca^{2+} influx and intracellular Ca^{2+} release, whereas α_2 -receptor-mediated contractions in the CMCA appear to rely almost entirely on influx of Ca^{2+} from the extracellular medium. Furthermore, nifedipine was more potent in inhibiting NA-induced contractions in the CMCA than in the RMCA. The Ca^{2+} channels activated by α_2 -receptor stimulation in the CMCA were highly sensitive to nifedipine and appeared to have similar characteristics as the potential operated channels activated by K^+ depolarization. In the RMCA the Ca^{2+} entry pathway was partly resistant to nifedipine. Thus, the differential effects of

nifedipine on α_1 - and α_2 -receptor-mediated responses in the different arteries are probably not only attributable to a larger intracellular Ca^{2+} release in the RMCA than in the CMCA but also to differences between the arteries in the nifedipine sensitivity of the Ca^{2+} influx pathway utilized by NA.

Regional variations in mechanisms providing activator Ca^{2+} to the contractile proteins were compared between cerebral and mesenteric arteries from cat and rat. Nifedipine, verapamil and diltiazem were equally potent in relaxing the CMCA and the CMA contracted by K^+ . However, nimodipine was slightly more potent in the CMCA than in the CMA suggesting that this drug may have a certain selectivity for the cerebral arteries. As shown by the experiments with Ca^{2+} free medium, the intracellular Ca^{2+} stores releasable by NA appeared to be larger in mesenteric than in cerebral arteries. Although the tonic component of the NA-induced contraction was dependent on Ca^{2+} influx in all the vessels studied, nifedipine reduced this component considerably more in cerebral than in mesenteric arteries, suggesting that the Ca^{2+} entry pathway was more resistant to nifedipine in mesenteric than in cerebral arteries. Notably, the maximum nifedipine inhibition of the tonic component seemed to be correlated to the amplitude of the contraction elicited by NA in Ca^{2+} -free medium. This suggests that the nifedipine resistant part of the tonic component somehow is related to the extent to which NA releases intracellular Ca^{2+} in that vessel, i.e. the greater the intracellular Ca^{2+} release, the greater the nifedipine resistance of the tonic response.

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REFERENCES

- AHLQUIST, R.P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.* 153 :586-600.
- ALLEN, G.S. & BANGHART, S.B. (1979). Cerebral arterial spasm: Part 9. In vitro effects of nifedipine on serotonin-, phenylephrine-, and potassium-induced contractions of canine basilar and femoral arteries. *Neurosurg.* 4:37-42.
- AMANN, F.W., BOLLI, P., KIOWSKI, W. & BÜHLER, F.R. (1980). Verstärkte α -Adrenoceptor-vermittelte Vasokonstriktion bei essentieller Hypertonie. *Schweiz Med. Wochenschr.* 110:1941-1944.
- ANDERSSON, K.-E-. LARSSON, B. & SJÖGREN, C. (1984). Characterization of the α -adrenoceptors in the female rabbit urethra. *Br. J. Pharmacol.* 81:293-300.
- ARIËNS, E.J. & SIMONIS, A.M. (1976). Receptors and receptor mechanisms. Beta-adrenoceptor Blocking Agents, eds P.R. Saxena & R.P. Forsyth. North-Holland Publishing Company, Amsterdam.
- ARIËNS, E.J. & A.M. SIMONIS. (1983). Physiological and pharmacological aspects of adrenergic receptor classification. *Biochem. Pharmacol.* 32:1539-1545.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* 14:48-59.
- AWAD, R., PAYNE, R. & DETH, R.C. (1983). Alpha adrenergic receptor subtype associated with receptor binding, Ca^{++} influx, Ca^{++} release and contractile events in the rabbit aorta. *J. Pharmacol. Exp. Ther.* 227:60-67.
- BARNES, P., KARLINER, J., HAMILTON, C. & DOLLERY, C. (1979). Demonstration of α_1 -adrenoceptors in guinea pig lung using 3H -prazosin. *Life Sci.* 25:1207-1214.

BARNES, P.J. B.-E. SKOOGH, NADEL, J.A. & J.M. ROBERTS. (1983). Postsynaptic α_2 -adrenoceptors predominate over α_1 -adrenoceptors in canine tracheal smooth muscle and mediate neuronal and hormonal α -adrenergic contraction. *Mol. Pharmacol.* 23:570-575.

BENTLEY, S.M., DREW, G.M. & WHITING, S.B. (1977). Evidence for two distinct types of postsynaptic α -adrenoceptor. *Proceedings of the B.P.S.* July 1977, p116-117.

BERTHELSEN, S. & PETTINGER, W.A. (1977). A functional basis for classification of α -adrenergic receptors. *Life Sci.* 21:595-606.

BEVAN, J.A. (1983). Diltiazem selectively inhibits cerebrovascular extrinsic but not intrinsic myogenic tone. *Circ. Res.* (suppl 1) 52:104-109.

BEVAN, J.A. (1984). "The γ -connection": are we ready to throw out the α -adrenoceptor in sympathetic vasoconstriction?. *Trends Pharmacol. Sci.* Febr. p53-55.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Pharmacol. Rev.* 59:606-718.

BOU, J., LLENAS, J. & MASSINGHAM, R. (1983). Calcium entry blocking drugs, "calcium antagonists" and vascular smooth muscle function. *J. Auton. Pharmac.* 3:219-232.

BRANDT, L., ANDERSSON, K.-E., EDVINSSON, L. & LJUNGGREN, B. (1981). Effects of extracellular calcium and of calcium antagonists on the contractile responses of isolated human pial and mesenteric arteries. *J. Cerebr. Blood Flow Metabol.* 1:339-347.

BRODDE, O.-E., HARDUNG, A., EBEL, H. & BOCK, K.D. (1982). GTP regulates binding of agonists to α_2 -adrenergic receptors in human platelets. *Arch. Int. Pharmacodyn.* 258:193-207.

BROWN, G.L. & GILLESPIE, J.S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol.* 138:81-102.

CASTEELS, R. & DROOGMANS, G. (1981). Exchange characteristics of the noradrenaline sensitive calcium store in vascular smooth muscle cells of rabbit ear artery. *J. Physiol. (Lond.)* 317:263-279.

CAUVIN, C., LOUTZENHISER, R., AWANG, O. & VAN BREEMEN, C. (1982a). α_1 -adrenoceptors induce Ca^{2+} influx and intracellular Ca^{2+} release in isolated rabbit aorta. *Eur. J. Pharmacol.* 84:233-235.

CAUVIN, C., LOUTZENHISER, R. & VAN BREEMEN, C. (1983). Mechanisms of calcium antagonist-induced vasodilation. *Ann. Rev. Pharmacol. Toxicol.* 23:373-396.

CAUVIN, C., SAIDA, K. & VAN BREEMEN, C. (1982b). Effects of Ca^{2+} antagonists on Ca^{2+} fluxes in resistance vessels. *J. Cardiovasc. Pharmacol. (Suppl)* 4:287-290.

CAVERO, I., FENARD, S., GOMENI, G., LEFEVRE, F. & ROACH, A.G. (1978). Studies on the mechanisms of the vasodilator effects of prazosin in dogs and rabbits. *Eur. J. Pharmacol.* 49:259-270.

CAVERO, I., SHEPPERSON, N., LEFEVRE-BORG, F. & LANGER, S.Z. (1983). Differential inhibition of vascular smooth muscle responses to α_1 - and α_2 -adrenoceptor agonists by diltiazem and verapamil. *Circ. Res. (Suppl 1)* 52:69-76.

COLUCCI, W.S., GIMBRONE, JR. M.A. & ALEXANDER, R.W. (1980). Characterization of postsynaptic α -adrenergic receptors by ^3H -dihydroergocryptine binding in muscular arteries from the rat mesentery. *Hypertension* 2:149-155.

COLUCCI, W.S., GIMBRONE, JR. M. A. & ALEXANDER, R.W. (1981). Regulation of the postsynaptic α -adrenergic receptor in rat mesenteric artery. Effects of chemical sympathectomy and epinephrine treatment. *Circ. Res.* 48:104-111.

CONSTANTINE, J.W., GUNNELL, D. & WEEKS, R.A. (1980). α_1 - and α_2 -vascular adrenoceptors in the dog. *Eur. J. Pharmacol.* 66:281-286.

DALE, H.H. (1906). On some physiological actions of ergot. *J. Physiol.* 34:163-206.

DALE, H.H. (1914). The action of certain esters and ethers of choline and their relation to muscarine. *J. Pharmacol. Exp. Ther.* 6:147-193.

DE MEY, J. & VANHOUTTE, P.M. (1981). Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ. Res.* 48:875-884.

DETH, R. & VAN BREEMEN, C. (1974). Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta. *Pflügers Arch.* 348:13-22.

DOCHERTY, J.R. & STARKE, K. (1981). Postsynaptic α -adrenoceptor subtypes mediating nerve-evoked contractions in rabbit blood vessels in vitro. *Proceedings of the B.P.S.* p 803.

DOCHERTY, J.R. & STARKE, K. (1982). An examination of the pre- and postsynaptic α -adrenoceptors involved in neuroeffector transmission in rabbit aorta and portal vein. *Br. J. Pharmacol.* 76:327-335.

DREW, G.M. (1978). Pharmacological characterization of the presynaptic α -adrenoceptors regulating cholinergic activity in the guinea-pig ileum. *Br. J. Pharmacol.* 64:293-300.

EBASHI, S. (1980). Regulation of muscle contraction. *Proc. R. Soc. Lond.* 207:259-286.

EDVINSSON, L., HARDEBO, J.-E. & OWMAN, CH. (1978). Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. *Circ. Res.* 42:143-151.

ELLIOTT, T.R. (1905). The action of adrenalin. *J. Physiol.* 32:401-467.

ELLIOTT, H.L. & REID, J.L. (1983). Evidence for postjunctional vascular α_2 -adrenoceptors in peripheral vascular regulation in man. *Clin. Sci.* 65:237-241.

FERRY, N., GOODHARDT, M., HANOUNE, J. & SEVENET, T. (1983). Binding of yohimbine stereoisomers to α -adrenoceptors in rat liver and human platelets. *Br. J. Pharmacol.* 78:359-364

FLAVAHAN, N.A., MCGRATH, J.C. (1984). Are human α -adrenoceptors atypical? *J. Cardiovasc. Pharmacol.* 6:208-210.

FORSTER, C. & WHALLEY, E.T. (1982). Analysis of the 5-hydroxytryptamine induced contraction of the human basilar arterial strip compared with the rat aortic strip in vitro. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 319:12-17.

FRANKHUYZEN, A.L. & MULDER, A.H. (1982). Pharmacological characterization of presynaptic α -adrenoceptors modulating ^3H noradrenaline and ^3H 5-hydroxytryptamine release from slices of the hippocampus of the rat. *Eur. J. Pharmacol.* 81:97-106.

FUDER, H., MUSCHOLL, E. & SPEMANN, R. (1983). The determination of presynaptic pA_2 values of yohimbine and phentolamine on the perfused rat heart under conditions of negligible autoinhibition. *Br. J. Pharmacol.* 79:109-119.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: *Handbook of Experimental Pharmacology*, vol 33 (ed H Blaschko & E. Muscoll), pp 283-355. Springer Verlag. New York.

GARDINER, J.C. & PETERS, C.J. (1982). Postsynaptic α_1 - and α_2 -adrenoceptor involvement in the vascular responses to neuronally released and exogenous noradrenaline in the hindlimb of the dog and cat. *Eur. J. Pharmacol.* 84:189-198.

GLUSA, E. & MARKWARDT, F. (1983). Characterization of postjunctional α -adrenoceptors in isolated human femoral veins and arteries. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 323:101-105.

GODFRAIND, T. & MILLER, R.C. (1983). Specificity of action of Ca^{2+} entry blockers. A comparison of their actions in rat arteries and in human coronary arteries. *Circ. Res. (Suppl 1)*. 52:81-91.

GODFRAIND, T., MILLER, R.C. & LIMA, S. (1983). Effects of yohimbine, rauwolscine and corynanthine on contractions and calcium fluxes induced by depolarization and prostaglandin $\text{F}_{2\alpha}$ in rat aorta. *Br. J. Pharmacol.* 80:115-121.

GREENGRASS, P. & BREMNER, R. (1979). Binding characteristics of ^3H -prazosin to rat brain α -adrenergic receptors. *Eur. J. Pharmacol.* 55:323-326.

HARDER, D.R., ABEL, P.W. & HERMSMEYER, K. (1981). Membrane electrical mechanisms of basilar artery constriction and pial artery dilation by norepinephrine. *Circ. Res.* 49:1237-1242.

HARDER, D.R. (1983). Heterogeneity of membrane properties in vascular smooth muscle cells from various vascular beds. *Fed. Proc.* 42:253-256.

HICKS, P.E. (1981). Antagonism of pre and postsynaptic α -adrenoceptors by BE 2254 (HEAT) and prazosin. *J. Auton. Pharmacol.* 1:391-397.

HIRST, G.D.S. & NEILD, T.O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature* 283:767-768.

HOFFMAN, B.B., LAVIN, T.N., LEFKOWITZ, R.J. & RUFFOLO, R. Jr. (1981). Alpha adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. *J. Pharmacol. Exp. Ther.* 219:290-295.

HOFFMAN, B.B. & LEFKOWITZ, R.J. (1980). An assay for alpha-adrenergic receptor subtypes using ^3H dihydroergocryptine. *Biochem. Pharmacol.* 29:452-454.

HORN, P.T., KOHLI, J.D., LISTINSKY, J.J. & GOLDBERG, L. I. (1982). Regional variation in alpha adrenergic receptors in the canine resistance vessels. *Naunyn Schmiedeberg's Arch. Pharmacol.* 318:116-172.

HÖGESTÄTT, E.D. & ANDERSSON, K.-E. (1984). On the postjunctional α -adrenoceptors in cerebral and mesenteric arteries from rat. Submitted for publication.

KENAKIN, T.P. (1982). The Schild regression in the process of receptor classification. *Can. J. Physiol. Pharmacol.* 60:249-265.

KOBINGER, W. & PICHLER, L. (1980). Investigation into different types of post- and presynaptic α -adrenoceptors at cardiovascular sites in rats. *Eur. J. Pharmacol.* 65:393.

KOPIN, I.J., BERV, K.R. & WEBB, J.G. (1973). Organ culture of sympathetic ganglia. *Frontiers in Catecholamine Research* p87-89. Ed: E. Usdin & S.H. Snyder. New York.

LAMAR, J.C. & EDVINSSON, L. (1980). 5-Hydroxytryptamine receptors. Contractile activity and mode of inhibition by methysergide in mammalian intracranial and extracranial vessels. *Arch. Int. Pharmacodyn.* 243:245-254.

LANDS, A.M., ARNOLD, A., MCAULIFF, J.P., LUDUENA, F.P. & BROWN, T.G., Jr. (1967). Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 214:597-598.

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmacol.* 23:1793-1800.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmacol.* 60:481-497.

LANGER, S.Z. (1980). Presynaptic receptors and modulation of neurotransmission: pharmacological implications and therapeutic relevance. Trends Neurosci. May p110-112.

LANGER, S.Z. (1981). Presynaptic regulation of the release of catecholamines. Pharmacol. Rev. 32:337-362.

LANGER, S.Z., MASSINGHAM, R. & SHEPPERSON, N.B. (1980). Presence of postsynaptic α_2 -adrenoceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb. Clin. Sci. 59:225-228.

LANGER, S.Z. & SHEPPERSON, N.B. (1981). Subclassification of α -adrenoceptors into α_1 - and α_2 -categories: relevance to antihypertensive therapy. In: New trends in arterial hypertension. Intern Symposium No 17 (ed M. Worcel et al.) pp 73-85. Elsevier/North Holland Biomedical Press.

LANGER, S.Z. & SHEPPERSON, N.B. (1982). Recent developments in vascular smooth muscle pharmacology: the post-synaptic α_2 -adrenoceptor. Trends Pharmacol. Sci. November p 440-444.

LANGLEY, J.N. (1905). On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. J. Physiol. 33:374-413.

LAVIN, T.N., HOFFMAN, B.B. & LEFKOWITZ, R.J. (1981). Determination of subtype selectivity of alpha-adrenergic antagonists. Comparison of selective and nonselective radioligands. Mol. Pharmacol. 20:28-34.

LLENAS, J. & MASSINGHAM, R. (1983). A comparison of the effects of verapamil and cinnarizine upon responses elicited by selective α_1 - and α_2 -adrenoceptor agonists in the autoperfused canine hindlimb. Eur. J. Pharmacol. 87:53-59.

MACKENZIE, E.T., EDVINSSON, L., ROBERT, J.-P., SKÄRBY, T. & YOUNG, A.R. (1983). Cerebrovascular responses to haemorrhagic hypotension in anaesthetized cats: Effects of selective α_1 - (prazosin) and α_2 - (yohimbine) adrenoceptor antagonists. *J. Cerebr. Blood Flow Metabol.* (suppl 1). 3:654-655.

MADJAR, H., DOCHERTY, J.R. & STARKE, K. (1980). An examination of pre- and postsynaptic α -adrenoceptors in the autoperfused rabbit hindlimb. *J. Cardiovasc. Pharmacol.* 2:619-627.

MANZINI, S., MAGGI, C.A. & MELI, A. (1983). α -adrenoceptor subtypes and Ca^{2+} mobilization the rabbit ear artery. *J. Pharm. Pharmacol.* 35:584-589.

MCGRATH, J.C. (1982). Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem. Pharmacol.* 31:467-484.

MCGRATH, J.C., FLAVAHAN, N.A. & MCKEAN, C.E. (1982). α_1 - and α_2 -adrenoceptor-mediated pressor and chronotropic effects in the rat and rabbit. *J. Cardiovasc. Pharmacol.* 4:101-107.

MEDGETT, I.C. & LANGER, S.Z. (1983). Characterization of smooth muscle alpha-adrenoceptors and of responses to electrical stimulation in the cat isolated perfused middle cerebral artery. *Naunyn Schmiedeberg's Arch. Pharmacol.* 323:24-32.

MINNEMANN, K.P., FOX, A.W. & ABEL, P.W. (1983). Occupancy of α_1 -adrenergic receptors and contraction of rat vas deferens. *Molecul. Pharmacol.* 23:359-368.

MOTULSKY, H.J. & INSEL, P.A. (1982). ^3H dihydroergocryptine binding to alpha-adrenergic receptors of human platelets. A reassessment using the selective radioligands ^3H prazosin, ^3H yohimbine, and ^3H rauwolscine. *Biochem. Pharmacol.* 31:2591-2597.

MOULDS, R.F.W. & JAUERNIG, R.A. (1977). Mechanism of prazosin collapse. *The Lancet* 22:200-201.

- NEILD, T.O. & HIRST, G.D.S. (1984). "The γ -connection": a reply. Trends Pharmacol. Sci. February p56-57.
- NEILD, T.O. & ZELCER, E. (1982). Noradrenergic neuromuscular transmission with special reference to arterial smooth muscle. Prog. Neurobiol. 19:141-158.
- OLIVER, G. & SCHÄFER, E.A. (1895). The physiological effects of extracts of the suprarenal capsules. J. Physiol. 18:230-276.
- PACIOREK, P.M. & SHEPPERSON, N.B. (1983). A study of pre- and postsynaptic α_2 -adrenoceptors in the pithed rat. Br. J. Pharmacol. (Suppl) 79:225P.
- PEART, W.S. (1949). The nature of splenic sympathin. J. Physiol. 108:491-501.
- REICHENBACHER, D., REIMANN, W. & STARKE, K. (1982). α -adrenoceptor-mediated inhibition of noradrenaline release in rabbit brain cortex slices. Receptor properties and role of the biophase concentration of noradrenaline. Naunyn-Schmiedeberg's Arch. Pharmacol. 319:71-77.
- RUFFOLO, R.R. (1982). Important concepts of receptor theory. J. Auton. Pharmac. 2:277-295.
- RUFFOLO, R.R., Jr. (1983). Alpha-adrenoceptors. In: Neuroreceptors in Health & Disease, ed. J. Marwaha & W. Andersson. S. Karger AG, Basel.
- RUFFOLO, R.R., Jr., YADEN, E.L. & WADDELL, J.E. (1980). Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha adrenergic receptor subtypes in tissues from the rat. J. Pharmacol. Exp. Ther. 213:557-561.

- RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. (1981). Postsynaptic alpha-adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of alpha-2 adrenergic receptors in rat aorta. *J. Pharmacol. Exp. Ther.* 217:235-240.
- RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. 1982. Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *J. Pharmacol. Exp. Ther.* 221:309-314.
- SAKAKIBARA, Y., FUJIWARA, M. & MURAMATSU, I (1982). Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. *Naunyn Schmiedeberg's Arch. Pharmacol.* 319:1-7.
- STARKE, K. (1981). α -Adrenoceptor subclassification. *Rev. Physiol. Biochem. Pharmacol.* 88:199-236.
- STARKE, K. (1981). Presynaptic receptors. *Ann. Rev. Pharmacol. Toxicol.* 21:7-30.
- STARKE, K. & DOCHERTY, J.R. (1980). Recent developments in α -adrenoceptor research. *J. Cardiovasc. Pharmacol. (Suppl 3)* 2:269-286.
- STARKE, K. & DOCHERTY, J.R. (1983). Presynaptic receptors in noradrenergic transmission in the pulmonary artery of the rabbit. *Vascular Neuroeffector Mechanisms: 4th International Symposium*, ed. J.A. Bevan et al., Raven Press, New York, p 103-109.
- STARKE, K., ENDO, T., TAUBE, H.D. (1975). Relative pre and postsynaptic potencies of α -adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedeberg's Arch Pharmacol.* 291:55-78.
- STARKE, K. & LANGER, S.Z. (1979). A note on terminology for presynaptic receptors. In: *Presynaptic receptors*. Ed. S.Z. Langer, K.Starke & M.L. Dubocovich, Pergamon Press, Oxford, p 1-3.

STEEN, S., SJÖBERG, T., SKÄRBY, T., NORGRÉN, L. & ANDERSSON, K.-E. (1984). Postjunctional α_1 - and α_2 -adrenoceptors mediating contraction in isolated groin arteries and veins. *Acta Physiol Scand*. Accepted for publication.

STEVENS, M.J. & MOULDS, R.F.W. (1981). Heterogeneity of post-junctional α -adrenoceptors in human vascular smooth muscle. *Arch. Int. Pharmacodyn.* 254:43-57.

STEINSLAND, O.S., FURCHGOTT, R.F. & KIRPEKAR, S.M. (1973). Biphasic vasoconstriction of the rabbit ear artery. *Circ. Res.* 32:49-58.

SULLIVAN, A.T. & DREW, G.M. (1980). Pharmacological characterization of pre- and postsynaptic α -adrenoceptors in dog saphenous vein. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 314:249-258.

TAKAMINE, J. (1901). The isolation of the active principle of the suprarenal gland. *J. Physiol. Lond.* 27:29-30.

THARP, M.D., HOFFMAN, B.B. & LEFKOWITZ, R.J. (1981). α -Adrenergic receptors in human adipocyte membranes: direct determination by ^3H yohimbine binding. *J. Clin Endocrinol. Metabol.* 52:709-714.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1980). Vasoconstriction mediated by postsynaptic α_2 -adrenoceptor stimulation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 313:17-20.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P. A. (1981). The postsynaptic α_2 -adrenoceptor. *J. Auton. Pharmacol.* 1:171-183.

TODA, N. (1983). Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. *J. Pharmacol. Exp. Ther.* 226:861-868.

TOWART, R. (1981). The selective inhibition of serotonin-induced contractions of rabbit cerebral vascular smooth muscle by calcium-antagonistic dihydropyridines. An investigation of the mechanisms of actions of nimodipine. *Circ. Res.* 48:650-657.

TOWART, R., WEHINGER, E., MEYER, H. & KAZDA, S. (1982). The effects of nimodipine, its optical isomers and metabolites on isolated vascular smooth muscle. *Arzneim-Forsch. Drug Res.* 32:338-346.

VAN BRUMMELEN, P., VERMEY, P., TIMMERMAN, P.B.M.W.M. & VAN ZWIETEN, P.A. (1982). Preliminary evidence for postsynaptic α_2 -adrenoceptor in the vasculature of the human forearm. *Proceedings of the B.P.S.*, September 1982, 134P-135P.

VAN MEEL, J.C.A. (1982). The vascular postjunctional α_2 -adrenoceptor. Thesis. University of Amsterdam.

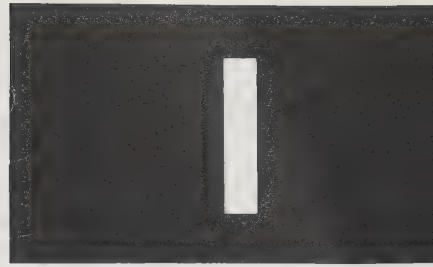
VAN MEEL, J.C.A., DE JONGE, A., KALKMAN, H.O., WILFFERT, B., TIMMERMAN, P.B.M.W.M. AND VAN ZWIETEN, P.A. (1981). Organic and inorganic calcium antagonists reduce vasoconstriction in vivo mediated by postsynaptic α_2 -adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 316:288-293.

VAN MEEL, J.C.A., TIMMERMAN, P.B.M.W.M. & VAN ZWIETEN, P.A. (1983). α_1 - and α_2 -adrenoceptor stimulation in the isolated perfused hindquarters of the rat: An in vitro model. *J. Cardiovasc. Pharmacol.* 5:580-585.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.* 143:299-330.

VAN ZWIETEN, P.A., VAN MEEL, J.C.A. & TIMMERMAN, P.B.M.W.M. (1982). Calcium antagonists and α_2 -adrenoceptors. Possible role of extracellular calcium ions in α_2 -adrenoceptor-mediated vasoconstriction. *J. Cardiovasc. Pharmacol.* (suppl) 4:273-279.

- VON EULER, U.S. (1946). The presence of a substance with sympathin E properties in spleen extracts. *Acta Physiol. Scand.* 11:168-186.
- VON EULER, U.S. (1948). Identification of the sympathomimetic ergone in adrenergic nerves of cattle (sympathin-N) with laevo-noradrenaline. *Acta Physiol. Scand* 16:63-74.
- VON EULER, U.S. (1951). The nature of adrenergic nerve mediators. *Pharmac. Rev.* 3:247-277.
- WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 308:127-136.
- WALUS, K.M., FONDACARO, J.D. & JACOBSON, E.D. (1981). Effects of calcium and its antagonists on the canine mesenteric circulation. *Circ. Res.* 48:692-700.
- WEISS, R.J., WEBB, R.C. & SMITH, C.B. (1983). Alpha-2 adrenoceptors on arterial smooth muscle: Selective labeling by ³H clonidine. *J. Pharmacol. Exp. Ther.* 225:599-605.
- WIKBERG, J.E.S. (1978). Pharmacological classification of adrenergic α -receptors in the guinea pig. *Nature* 273:164-166.
- WIKBERG, J.E.S. (1979). The pharmacological classification of adrenergic α_1 - and α_2 -receptors and their mechanisms of action. *Acta Physiol. Scand.* 68, suppl 468.



Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries

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The vascular α -adrenoceptors in isolated feline cerebral, lingual and mesenteric arteries were characterized and compared. In the middle cerebral artery the relative order of potency for agonists was: clonidine > oxymetazoline > noradrenaline > phenylephrine which indicates that the postjunctional α -receptor in this vessel is of α_2 -type. This view is further supported by the finding that yohimbine, but not prazosin, had a potent, mainly competitive blocking action. In peripheral arteries, clonidine was without effect. In these vessels, the potency difference between phenylephrine and oxymetazoline was more than 40 times less than in cerebral vessels. The pA_2 -values for prazosin correlated well with pA_2 -values found for the interaction of this drug with α_1 -receptors in a variety of other tissues, thus suggesting the occurrence of an α_1 -receptor in these arteries. However, the pA_2 -values for yohimbine and rauwolscline correlated well with an α_2 -receptor, suggesting also the presence of α_2 -receptors. Schild plots for prazosin and rauwolscline in lingual arteries displayed slopes significantly lower than unity, which also supports the view of a mixture of α_1 - and α_2 -receptors in these vessels. However, the Schild plots for the antagonists in mesenteric arteries did not differ significantly from unity, a finding possibly indicating the presence of an α -receptor unable to differentiate between substances that in other tissues act preferentially on α_1 - or α_2 -receptors.

Key words: α -Adrenoceptor subtypes, cerebral arteries, peripheral arteries, cat, pA_2 -values

The existence of pre- and postjunctionally located α -adrenoceptors with different pharmacological characteristics was pointed out by Langer (1974). He suggested that the former should be called α_2 and the latter α_1 . Further investigations confirmed these differences, but also showed that α -receptors with the same pharmacological characteristics as α_2 -receptors were found in, e.g., platelets, cholinergic neurons and postjunctionally in vascular smooth muscle. It has therefore been widely accepted, that α -receptors should be subclassified pharmacologically and not dependent on anatomical localization or function (for reviews, see Langer & Shepperson 1981, Starke 1981). It now seems that α_1 - and α_2 -receptors are general subtypes and

that they may both occur in the same tissue. It may therefore be possible to selectively influence, e.g., different vascular regions by α -receptor agonists and antagonists, if the populations of α -receptors in the regions differ or one kind of α -receptor predominates. Previous studies in vitro have shown that the α -receptor in feline cerebral arteries is 'unusual' (Edvinsson & Owman 1974). Based on in vivo observations (Drew & Whiting 1979, Patel et al. 1981), it may be suggested that both α_1 - and α_2 -receptors occur postjunctionally in peripheral arteries from cat (Starke 1981).

The present study was performed in order to characterize and compare the α -receptor populations in cat cerebral and some peripheral arteries

using selective agonists and antagonists. In preliminary form, some of the present results have been published previously (Skärby et al. 1981).

METHODS

Procedure. About 50 cats of either sex were decapitated under sodium barbiturate anesthesia (Mebumal 30–40 mg/kg i.p.). The brain was quickly removed and put into cold buffer solution (composition, see below). Subsequently also the tongue and part of the mesenterium were taken out and immersed into buffer solution. Parts of middle cerebral, jejunal, and deep lingual arteries (outer diameter in situ 0.4–0.6 mm) were instantly taken out and dissected free from surrounding tissue. Care was taken to avoid stretching or other type of injury to the vessels. One to two mm long vessel segments were suspended between two L-shaped metal holders (0.2 mm in diameter) one of which was attached to a Grass polygraph for continuous recording of isometric tension. The position of the other holder could be changed by means of a movable unit allowing fine adjustments of vessel tension by varying the distance between the metal prongs. For further experimental details, see Högestätt et al. (1982).

The preparations were immersed in 5 ml temperature-controlled organ baths containing a modified Krebs solution of the following composition (in mM): NaCl 119, NaHCO₃ 15, KCl 4.6, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2, glucose 11. Cocaine 10⁻⁶ M and propranolol 10⁻⁷ M were also added. The bath solution was continuously aerated with a mixture of 5% CO₂ and 95% O₂ giving a pH of 7.4. The temperature was maintained at 37°C.

After mounting, an initial tension of 5 mN was applied to the vessels. This resulted in a spontaneous relaxation which was compensated for by frequent stretches in order to maintain a tension of 4 mN. This tension level was chosen, because in separate experiments on the middle cerebral artery maximum contractile force was obtained at a passive tension of between 1–5 mN. It was also observed that the maximum contractile force in lingual arteries was obtained at a passive tension of 2–9 mN. As the mesenteric arteries were of similar dimensions we assumed that a passive tension of 4 mN would be suitable also for these vessels.

After approximately 1½ h, when a steady level of 4 mN was reached, the contractile capacity of the vessels was examined by exposure to a potassium-rich buffer solution containing (in mM): KCl 127, NaHCO₃ 15, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2, glucose 11.

Concentration-response (cr) curves. After two reproducible contractions by potassium 127 mM were obtained (variation less than 10%), noradrenaline (NA) was added cumulatively until two reproducible concentration-response (cr) curves were accomplished. Preparations failing to produce reproducible contractions to potassium and NA were rejected. Vessels in which E_{max} (see below) of the NA cr-curve was less than 20% of the potassium induced contraction were also excluded.

After obtaining reproducible cr-curves to NA, also other agonists were added cumulatively. In the rare cases where more than one agonist (beside NA) was examined

on the same segment, the reproducibility of the NA cr-curve was confirmed by another cumulative addition of NA prior to the next addition of agonist. When an antagonist was examined the drug was added in its lowest concentration 15 minutes before and was present during the cumulative administration of NA. After repeated washing of the preparation, the next concentration of antagonist was tested in an identical manner.

Because of the pronounced tachyphylaxis observed with phenylephrine the experimental procedure for testing the antagonistic action of rauwolscine towards this agonist, was somewhat different. Five segments from the same vessel were studied simultaneously. After two reproducible potassium contractions three segments were exposed to different concentrations of rauwolscine whereas two segments served as controls. The mean EC₅₀-value for phenylephrine in the two control segments served as reference to which the EC₅₀-values of the other segments in the same experiment were compared when calculating the concentration ratios for the pA₂-determinations (see below).

The potassium-induced contraction was used as an internal standard in each vessel, and each contraction was expressed as percentage of the potassium-induced response. Separate experiments showed that phenolamine (10⁻⁶ M) reduced the potassium contraction with approximately 10% in each type of vessel, an effect that was only partially reduced by incubating the vessels in 10⁻⁶ M 6-hydroxydopamine for 12 h. This suggests that only a minor portion of the K⁺-response is mediated by released NA. As this component was similar in all vessel types the potassium-induced contraction was considered a suitable reference. Cocaine, 10⁻⁶ M, (for blockade of neuronal uptake) and propranolol, 10⁻⁷ M, (to block beta-adrenoceptors) were always present in the buffer solution.

Preliminary experiments showed that an increase in cocaine or propranolol concentrations with a factor of ten did not potentiate the NA-response, and propranolol in a concentration of 10⁻⁶ M, even reduced the NA-response. Addition of normetanephrine (10⁻⁶ M) to block extraneuronal uptake (uptake₂) did not have any potentiating effect on the NA cr-curve, and 10⁻⁵ M of the drug also reduced E_{max}.

Control segments were frequently run in parallel with the segments receiving test substances. The NA cr-curves in these segments remained reproducible throughout the experiments.

Some segments were exposed to only one concentration of antagonist. In these preparations, the blockade was of the same magnitude as if the same concentration was one of three subsequent antagonist concentrations given to the segment.

Treatment of data

pA₂, the negative logarithm of the antagonist concentration that displaces the agonist cr-curves towards higher concentrations with a factor of 2, was determined according to Arunlakshana & Schild (1959). EC₅₀, i.e. the concentration at which 50% of maximum contraction is obtained, was calculated for those cr-curves that were used for pA₂ determination.

All cr-curves were plotted graphically and E_{max}, i.e.

Table 1. The effects of α -receptor agonists in cat cerebral, lingual and mesenteric arteries
The values are given as mean $\pm$ SE and z is the number of observations

Artery	Agonist				
	Clonidine	Oxymetazoline	Noradrenaline	Phenylephrine	Methoxamine
Cerebral					
pD ₂	7.37 $\pm$ 0.10	7.24 $\pm$ 0.13	6.19 $\pm$ 0.08	$\leq$ 4.63	
E _{max}	12 $\pm$ 2	35 $\pm$ 4	35 $\pm$ 2	$\geq$ 23	
	$z=7$	$z=7$	$z=23$	$z=7$	
Lingual					
pD ₂	No effect	6.90 $\pm$ 0.13	5.76 $\pm$ 0.05	5.92 $\pm$ 0.24	5.33 $\pm$ 0.17
E _{max}		75 $\pm$ 8	99 $\pm$ 2	90 $\pm$ 8	70 $\pm$ 10
		$z=7$	$z=32$	$z=7$	$z=6$
Mesenteric					
pD ₂	No effect	6.74 $\pm$ 0.4	6.04 $\pm$ 0.04	5.89 $\pm$ 0.11	5.46 $\pm$ 0.07
E _{max}		83 $\pm$ 9	108 $\pm$ 3	99 $\pm$ 5	92 $\pm$ 6
		$z=7$	$z=28$	$z=7$	$z=6$

the maximum contraction obtained with an agonist, and pD₂, i.e. the negative logarithm of the EC₅₀-value, were calculated. Student's *t*-test was used to determine statistical significance (the unpaired test being used if not otherwise stated). The regressions of the Schild plots, based on linear least square fit, and the *t*-test analyses in conjunction with these were performed on a calculator (Hewlett Packard 41 CV) by using a program which was kindly supplied by Dr Claus Rerup, Department of Pharmacology, University of Lund, Lund.

Drugs

Clonidine hydrochloride (ACO), DL-arterenol hydrochloride, L-phenylephrine hydrochloride, yohimbine hydrochloride, DL-normetanephrine hydrochloride (Sigma), methoxamine hydrochloride (Burroughs, Wellcome), oxymetazoline chloride (Draco), prazosin hydrochloride (Pfizer), propranolol hydrochloride (ICI), rauwolfscine hydrochloride (Roth). The drugs were dissolved in 0.9% NaCl containing 1.0 mM ascorbic acid except prazosin which was dissolved in 10% lactic acid. The concentrations below are given as final concentrations in the organ bath.

RESULTS

Effects of α -receptor agonists

There was a considerable variation in NA-response in the middle cerebral arteries from different cats, some vessels being unresponsive to the agent even if the peripheral arteries from the same cat responded well within the same concentration range. Such interindividual variation in responsiveness of cerebral arteries has been described previously in man (Brandt et al. 1981). All agonist-induced contractions, except the ones elicited by oxymetazoline, were easily washed out. Contractions induced by oxymetazoline were slow in onset and persisted for

at least 1 h despite frequent washing. NA was less potent ($p<0.001$) in lingual than in cerebral and mesenteric arteries in which it was equally potent (Table 1). When expressed as per cent of the potassium contraction, the intrinsic activity of NA decreased according to the following in the different vascular regions: mesenteric>lingual>cerebral arteries ($p<0.001$ for all differences). All agonist pD₂- and E_{max}-values are summarized in Table 1.

Cerebral arteries (Fig. 1). In the middle cerebral artery (MCA) oxymetazoline behaved as a full agonist, whereas clonidine displayed only approximately one third of the intrinsic activity of NA ($p<0.01$, paired *t*-test). However, when the curves for clonidine and oxymetazoline were plotted as per cent of their own maximum contractions the curves were almost identical. The curves for phenylephrine did not exhibit any sigmoidal relationship and no plateau was reached even at a concentration of 3×10^{-4} M phenylephrine. Therefore, neither E_{max}- nor pD₂-values could be accurately calculated for this drug. The order of potency was: clonidine>oxymetazoline>NA>phenylephrine.

Peripheral arteries. In the jejunal and deep lingual arteries clonidine was without effect in the concentrations used (10^{-8} – 10^{-5} M) whereas the other agonists produced concentration-related contractions. The order of potency was: oxymetazoline>NA>phenylephrine>methoxamine. Methoxamine ($p<0.05$) and oxymetazoline ($p<0.01$) were partial agonists when compared to NA, whereas phenylephrine was a full agonist (Fig. 2 and 3).

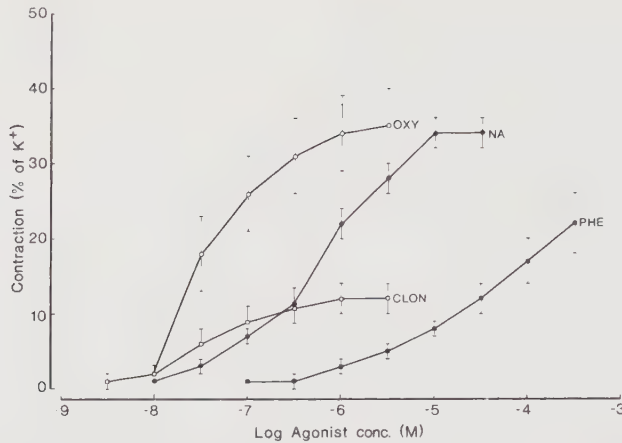


Fig. 1. The contractile responses to oxymetazoline (OXY), clonidine (CLON), noradrenaline (NA) and phenylephrine (PHE) in middle cerebral arteries. Responses are expressed as percentage of the potassium induced contraction and each point represents the mean $\pm$ SE of 7–22 expts.

Effects of α -receptor antagonists

Cerebral arteries. The middle cerebral artery displayed a high sensitivity towards yohimbine as demonstrated by a shift of the cr-curve to NA by low concentrations of the drug (10^{-8} M). However,

a statistically significant depression of $E_{\max}$ could be observed for both 10^{-8} M and 10^{-7} M of yohimbine ($p < 0.01$, paired t -test, Fig. 4a). As this indicated a non-competitive component in the antagonistic action of yohimbine, pA_2 could not be

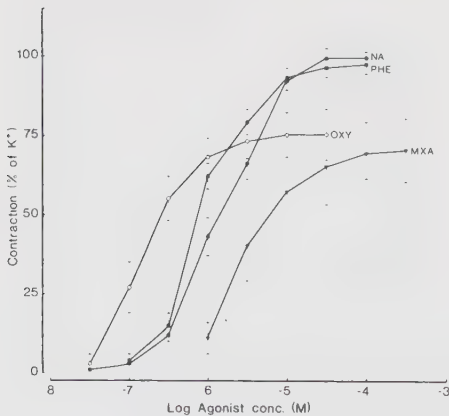


Fig. 2. The contractile responses to oxymetazoline (OXY), phenylephrine (PHE), noradrenaline (NA) and methoxamine (MXA) in deep lingual arteries. Responses are expressed as percentage of the potassium induced contraction and each point represents the mean $\pm$ SE of 6–30 expts.

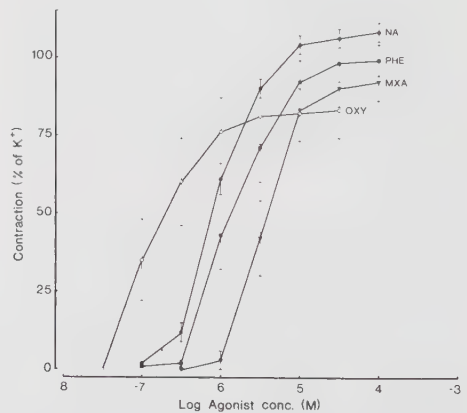


Fig. 3. Contractile responses to oxymetazoline (OXY), noradrenaline (NA), phenylephrine (PHE) and methoxamine (MXA) in mesenteric arteries. Responses are expressed as percentage of the potassium induced contraction and each point represents mean $\pm$ SE of 6–32 expts.

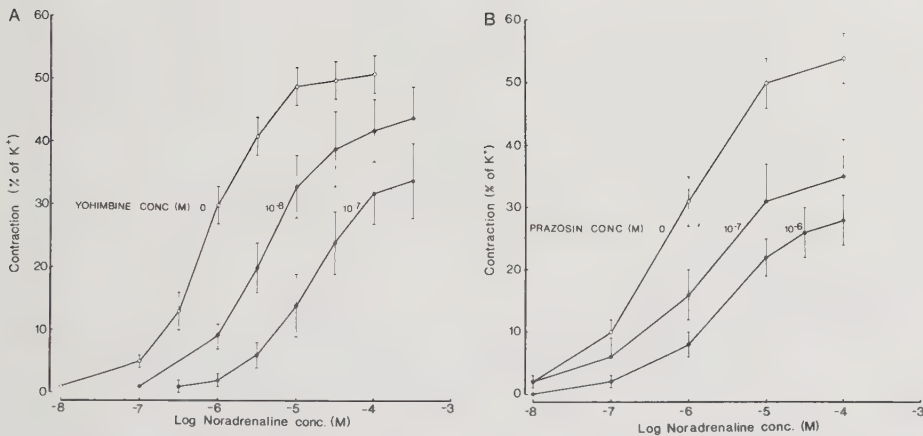


Fig. 4. Contractile responses to noradrenaline in middle cerebral arteries. (A) Antagonism by yohimbine. (B) Antagonism by prazosin. Responses are expressed as percentage of the potassium induced contraction and each point represents mean $\pm$ SE of 7–20 expts.

accurately determined. Prazosin was not able to produce a parallel shift of the cr-curve to NA. It did, however reduce $E_{\max}$ in the concentrations 10⁻⁷ M and 10⁻⁶ M ($p < 0.01$ and $p < 0.05$, paired t -test) (Fig. 4b).

Lingual arteries. In the deep lingual artery all three antagonists, prazosin, yohimbine and rauwolscine, were able to shift the cr-curve to NA in a parallel manner without depression of $E_{\max}$ (Fig. 5a, b, c).

When the yohimbine values were analysed according to Arunlakshana & Schild (1959) (Schild plot) a regression line was obtained with a slope not significantly different from unity. However, when the prazosin and rauwolscine results were analysed

by the same technique the slopes were significantly lower than unity ($p < 0.001$ for both) (Fig. 6a). The antagonistic action of rauwolscine was also tested with phenylephrine as agonist (Fig. 5d). The Schild plot for this antagonism was similar to the one obtained when NA was used as agonist (Fig. 6b) and revealed a slope factor significantly lower than unity ($p < 0.001$). Slope factors and pA_2 -values for the antagonists are all given in Table 2.

The slope factors of rauwolscine and prazosin indicate an antagonism not fulfilling the criteria mentioned by Furchgott (1972) to be necessary for competitive antagonism with one type of receptor.

Mesenteric arteries. In the jejunal arteries, prazosin, yohimbine and rauwolscine each displaced

Table 2. Potencies of α -receptor antagonists in cat lingual and mesenteric arteries

The values are given as mean $\pm$ SE and z is the number of points in the regressions

Artery	Antagonist	Agonist	z	Slope of regression line (95% confidence interval)	pA_2 -values (95% confidence interval)
Lingual	Prazosin	NA	26	0.81 (0.72–0.90)	8.76 (8.57–9.02)
Lingual	Yohimbine	NA	21	0.94 (0.78–1.10)	7.73 (7.57–7.95)
Lingual	Rauwolscine	NA	27	0.67 (0.56–0.77)	7.44 (7.27–7.66)
Lingual	Rauwolscine	Phenylephrine	19	0.74 (0.60–0.87)	7.42 (7.22–7.70)
Mesenteric	Prazosin	NA	29	0.93 (0.82–1.04)	8.70 (8.50–8.94)
Mesenteric	Yohimbine	NA	23	0.95 (0.77–1.12)	7.34 (7.16–7.56)
Mesenteric	Rauwolscine	NA	18	0.95 (0.81–1.08)	7.35 (7.20–7.54)
Mesenteric	Rauwolscine	Phenylephrine	24	0.88 (0.71–1.05)	7.39 (7.19–7.68)

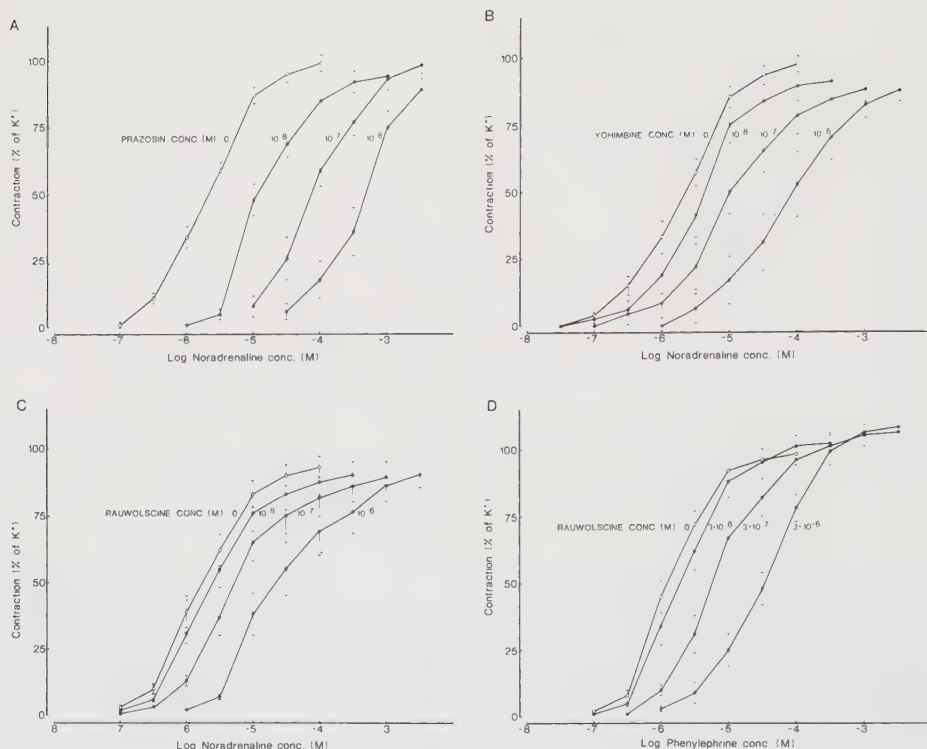


Fig. 5. Contractile responses in deep lingual arteries. (A) Concentration-response curve to noradrenaline in the presence of prazosin. (B) Concentration response curve to noradrenaline in the presence of yohimbine. (C) Concentration-response curve to noradrenaline in presence of rauwolfscine. (D) Concentration-response curve to phenylephrine in the presence of rauwolfscine. Responses are expressed as percentage of the potassium induced contraction and each point represents mean $\pm$ SE of 6–22 expts.

the NA cr-curve towards higher concentrations without depression of $E_{\max}$ (Fig. 7a, b, c). In Schild plots none of the slopes for the antagonists differed significantly from unity (Table 2, Fig. 8a).

Also in this preparation rauwolfscine was tested with phenylephrine as agonist. Increasing concentrations of rauwolfscine caused concentration-dependent parallel shifts of the cr-curves to phenylephrine (Fig. 7d). The Schild plot produced a slope and a pA_2 -value similar to those obtained with NA as agonist (Fig. 8b). All pA_2 -values and slope factors are given in Table 2.

Thus, in the jejunal arteries the slope factors for the interaction between prazosin, yohimbine or rauwolfscine and NA, or rauwolfscine and phenyl-

ephrine did not differ significantly from unity, indicating a competitive antagonism with one type of receptor by these drugs.

DISCUSSION

The selectivity of drugs stimulating or blocking α_1 - and α_2 -adrenoceptors and the criteria for the subclassification into these categories have recently been discussed in detail (see e.g. Wikberg 1979, Langer & Shepperson 1981, Starke 1981, Timmermans & Van Zwieten 1981). It was suggested that α -receptors which are most effectively activated by phenylephrine and methoxamine, and antagonized by prazosin should be classified as α_1 . The recep-

tors preferentially stimulated by clonidine, oxymetazoline, tramazolin and xylazine, and blocked by yohimbine and rauwolsine should be denominated α_2 . In vascular smooth muscle both types of α -adrenoceptor mediating constriction have been demonstrated (for reviews, see Starke 1981 and Timmermans & Van Zwieten 1981). In the present investigation this classification has been used for further characterization of α -receptors in different vascular regions in the cat.

Cerebral vessels

In the MCA, the relative order of potency for agonists was clonidine > oxymetazoline > NA > phenylephrine. This order of potency is the same as that proposed to be typical for α_2 -receptors (Langer 1981). Yohimbine distinctly displaced the NA cr-curve towards higher concentrations already at a concentration of 10^{-8} M (Fig. 4a).

The agonist pattern together with the potent blockade obtained by yohimbine indicate that the postjunctional α -receptor in this vessel is of α_2 -type. This is further supported by the finding that prazosin was not able to produce a parallel shift of the NA cr-curve towards higher concentrations, but rather caused a reduction of $E_{\max}$.

Experiments *in vivo* have been performed showing a constriction of pial arteries upon stimulation of sympathetic nerves in e.g. cat (see Edvinsson & MacKenzie 1977). This effect could be blocked with α -receptor blockers, which indicates that the NA released from sympathetic nerves interacts with the postjunctional α -receptors. As judged from the present results, these postjunctional α -receptors, apparently accessible to NA released from adrenergic nerves, seem to be of α_2 -type. Vascular α_2 -receptors were suggested to be located predominantly extrajunctionally in the dog hind limb and therefore not accessible to released NA (Langer et al. 1980). However, our observation in the MCA, and previous data from stimulation experiments *in vivo* do not support the general validity of this view.

Peripheral arteries

The relative order of agonist potency in lingual and mesenteric arteries was the same as that suggested by Langer & Shepperson (1981) and Starke (1981) to be representative for α_2 -receptors. However, clonidine was without effect, and oxymetazoline was only 7–10 times more potent than phenyleph-

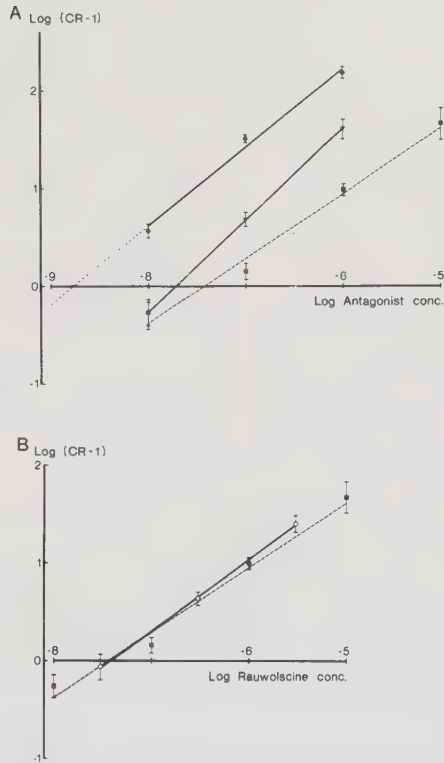


Fig. 6. Schild plots for lingual arteries. (A) Antagonism of prazosin (●), yohimbine (▼) and rauwolsine (■) with NA as agonist. (B) Antagonism of rauwolsine with NA (■) and phenylephrine (○) as agonists.

rine in these arteries. This should be compared with the findings in the MCA showing that clonidine was a potent (but partial) agonist and oxymetazoline was at least 400 times more potent than phenylephrine. Thus, when compared to cerebral arteries the peripheral arteries were less sensitive to agonists with preference for α_2 -receptors and more sensitive to agonists with preference for α_1 -receptors.

The difference between cerebral and peripheral arteries was even more pronounced when the results obtained with antagonists were compared. In contrast to its effect in cerebral arteries, prazosin was very effective in displacing the NA cr-curve towards higher concentrations in lingual and mesenteric vessels without any reduction in $E_{\max}$. The

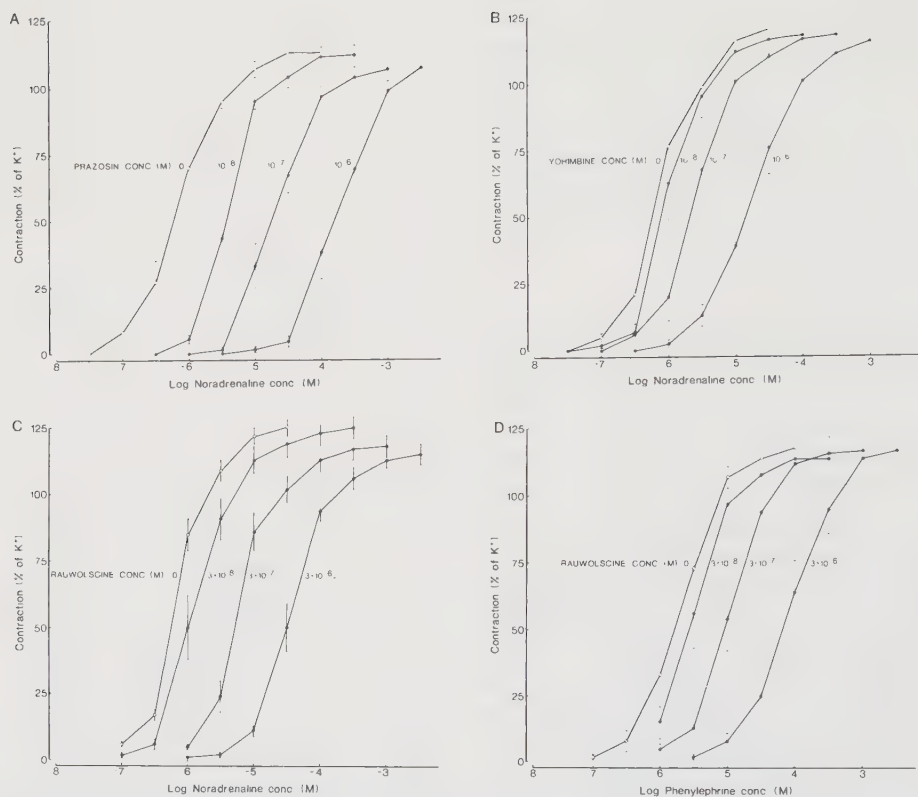


Fig. 7. Contractile response in mesenteric arteries. (A) Concentration-response curve to noradrenaline in the presence of prazosin. (B) Concentration-response curve to noradrenaline in the presence of yohimbine. (C) Concentration-response curve to noradrenaline in the presence of rauwolscine. (D) Concentration-response curve to phenylephrine in the presence of rauwolscine.

pA_2 -values for prazosin, 8.76 in lingual and 8.70 in mesenteric arteries, are comparable to pA_2 -values for the interaction between prazosin and α_1 -receptors in several tissues (Table 4) which suggests the occurrence of postjunctional α_1 -receptors in these peripheral arteries.

On the other hand our pA_2 -values for yohimbine in mesenteric and lingual arteries, 7.34 and 7.73, correlate best with pA_2 -values reported in the literature for the interaction of this drug with α_2 -receptors. These pA_2 -values range between 7.36 and 8.32 (Table 3). The pA_2 -values for the interaction between yohimbine and α_1 -receptors reported by other workers are in the range 6.10 to 6.56

(Table 3). Our pA_2 -value for rauwolscine with NA as agonist was 7.35 and 7.44 in mesenteric and lingual arteries, respectively. These values are similar to the EC_{30} -value for rauwolscine in causing increase in stimulation evoked 3H -noradrenaline overflow from rabbit pulmonary artery described by Weitzell et al. (1979), an effect considered to be mediated via α_2 -receptors.

The pA_2 -values for interaction of rauwolscine with postjunctional α -receptors (α_1) in rabbit pulmonary artery reported by Weitzell et al. (1979), and in rabbit blood vessels and rat anococcygeus muscle reported by Docherty & Starke (1981) range between 5.3 and 5.89. Thus, our pA_2 -values for

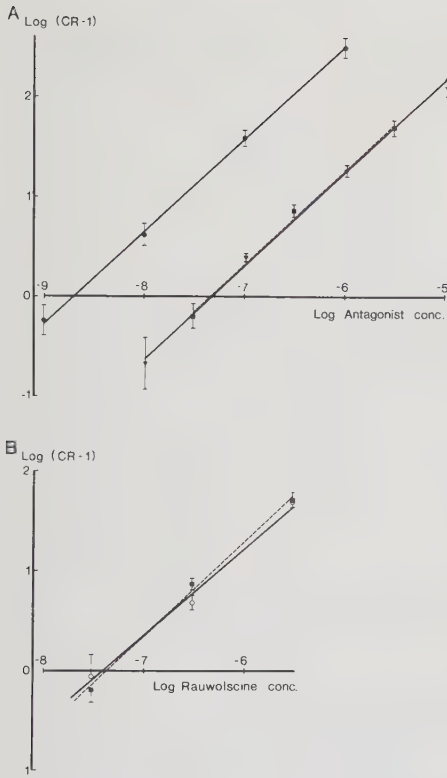


Fig. 8. Schild plots for mesenteric arteries. (A) Antagonism of prazosin (●), yohimbine (▼) and rauwolscine (■) with NA as agonist. (B) Antagonism of rauwolscine with NA (■) and phenylephrine (○) as agonists. Each point represents mean $\pm$ SE of 3–9 expts.

yohimbine and rauwolscine suggest the occurrence of an α_2 -receptor whereas those for prazosin would favour the occurrence of an α_1 -receptor in cat peripheral arteries.

If two postjunctional α -receptors are present in the same peripheral arteries it seems inappropriate to use the criteria for α -receptor classification proposed by Langer & Shepperson (1981) and Starke (1981), implying the *relative* order of potency of agonists and antagonists should be used to classify the receptor. In the present investigation we based our interpretation on the *absolute* potencies of antagonists (pA_2 -values) and compared these with representative studies in the literature.

Table 3. pA_2 -values reported in the literature for the interaction of yohimbine with α_1 - and α_2 -receptors in different tissues

pA_2	Tissue	Reference
<i>α_1-receptor</i>		
6.10	Rat vas deferens	Kapur & Mottram 1978
6.24	Rabbit pulmonary artery	Borowski et al. 1978
6.4	Rat anococcygeus muscle	Doxey et al. 1977
6.49	Rabbit uterus	Hoffman et al. 1981
6.56	Rabbit pulmonary artery	Weitzell et al. 1979
<i>α_2-receptors</i>		
7.36	Rat vas deferens	Drew 1977
7.4	Rat neocortex	Werner et al. 1979
7.78	Guinea-pig ileum	Drew 1978
8.18	Rat vas deferens	Doxey et al. 1977
8.32	Rat vas deferens	Kapur & Mottram 1978

In lingual arteries the slope factors for prazosin and rauwolscine in the Schild plots were significantly lower than unity. One explanation for this could be that both α_1 - and α_2 -receptors occur postjunctionally in these vessels. The reason why yohimbine had a slope factor not significantly different from unity may be that this substance, being the least selective of the three, was not able to distinguish between the two different types of receptor. In mesenteric arteries all antagonists displayed slope factors close to unity, indicating an interaction with only one type of receptor. Several mechanisms may have affected the slope factors, such as variations in uptake₁ and uptake₂, the influence of β -adrenoceptors or tachyphylaxis. However, control experiments were run making these possibilities unlikely (see Methods).

It can not be excluded that the drugs used also have intracellular actions, or that they interact with the receptors in an unusual manner and that these unpredicted actions could have influenced the results. Further studies with radioligand binding technique may, however, clarify some of these points.

Phenylephrine has been suggested to be relatively selective for α_1 -receptors (Borowski et al. 1977, Wikberg 1979). This view was supported by the low potency of the drug in cerebral arteries, where in the present study α_2 -receptors have been proposed to be predominant postjunctionally. Phenylephrine was at least 36 times less potent than NA in cerebral arteries whereas the two drugs were equipotent in lingual and mesenteric arteries.

Table 4. pA_2 -values reported in the literature for the interaction of prazosin with α_1 -receptors in different tissues

pA_2	Tissue	Reference
<i>α_1-receptors</i>		
8.2	Rat anococcygeus muscle	Doxey et al. 1977
8.2	Rabbit portal vein	Docherty & Starke 1981
8.4	Rabbit aorta	Docherty & Starke 1981
8.47	Rabbit aorta	Cavero et al. 1978
8.59	Rabbit aorta	Cavero et al. 1978
8.7	Rabbit pulmonary artery	Docherty & Starke 1981
8.8	Rat anococcygeus muscle	Docherty & Starke 1981
8.90	Rabbit uterus	Hoffman et al. 1981

Assuming that phenylephrine interacts with α_1 -receptors (indicated by its high pD_2 -value), and rauwolscine with α_2 -receptors (indicated by its high pA_2 -value), it could be expected that higher concentrations of rauwolscine would be necessary to achieve the same degree of displacement of the curve when phenylephrine was used instead of NA as agonist. However, this was not the case. In peripheral arteries there were no significant differences neither in pA_2 -value nor in slope factor if phenylephrine was used as agonist instead of NA. This is consonant with the finding that in mesenteric arteries the slope factors did not differ from unity for any of the antagonists used, suggesting an interaction with only one type of receptor. However, when the low slope factors in lingual arteries are taken into account, it rather suggests that phenylephrine is as unselective as NA in these arteries.

On the basis of the present data, the α -receptor in cat mesenteric arteries may be characterized as an α_1 -receptor with unusually high sensitivity for rauwolscine and yohimbine. However, the potency difference between prazosin and rauwolscine was in the present study approximately 20 times, whereas in a study by Docherty & Starke (1981), where α_1 -receptors in rabbit blood vessels and rat anococcygeus muscle were demonstrated, this potency difference was at least 1000 times. Postjunctionally in the rabbit pulmonary artery the potency difference between yohimbine and prazosin was approximately 200 times (calculated from Borowski et al. 1977, Weitzell et al. 1979, and Docherty & Starke 1981) whereas it was about 260 times in uterus from the same species (Hoffman et al. 1981). In both tissues the receptor was proposed to be of α_1 -type.

In feline mesenteric arteries as shown in the present study, the potency difference between these two drugs was approximately 20 times. In rat anococcygeus muscle Doxey et al. (1971) found a potency difference between prazosin and yohimbine of 63 times which also considerably exceeds the present values. Even if species differences are taken into account, it may be questioned whether it is appropriate to classify the α -receptor of cat mesenteric arteries as an α_1 -receptor.

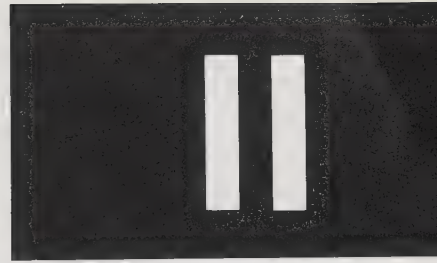
The present data thus suggest that in cat cerebral arteries the postjunctionally located α -receptor is mainly of α_2 -type. However, in mesenteric and lingual arteries the postjunctional α -receptors had properties characteristic of α_1 - as well as of α_2 -receptors. In lingual arteries this may be explained by the co-existence of α_1 - and α_2 -receptors, whereas the data from mesenteric arteries rather suggest the occurrence of an α -receptor unable to differentiate between substances that in other tissues act preferentially on α_1 - or α_2 -receptors.

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REFERENCES

- ARUNLAKSHANA, O. & SCHILD, H. O. 1959. Some quantitative uses of drug antagonists. *Br J Pharmacol* 14: 48–59.
- BOROWSKI, E., STARKE, K., EHRL, H. & ENDO, T. 1977. A comparison of pre- and postsynaptic effects of α -adrenolytic drugs in the pulmonary artery of the rabbit. *Neuroscience* 2: 285–296.
- BRANDT, L., LJUNGGREN, B., ANDERSSON, K.-E. & HINDFELT, B. 1981. Individual variation in response of human cerebral arterioles to vasoactive substances, human plasma and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg* 55: 431–437.
- CAVERO, I., FENARD, S., GOMENI, G., LEFEVRE, F. & ROACH, A. G. 1978. Studies on the mechanisms of the vasodilator effects of prazosin in dogs and rabbits. *Eur J Pharmacol* 49: 259–270.
- DOCHERTY, J. R. & STARKE, K. 1981. Postsynaptic α -adrenoceptor subtypes in rabbit blood vessels and rat anococcygeus muscle studied in vitro. *J Cardiovasc Pharmacol* 3: 854–866.
- DOXEY, J. D., SMITH, C. F. C. & WALKER, J. M. 1977. Selectivity of blocking agents for pre- and postsynaptic α -adrenoceptors. *Br J Pharmacol* 60: 91–96.
- DREW, G. M. 1977. Pharmacological characterization of the presynaptic α -adrenoceptor in the rat vas deferens. *Eur J Pharmacol* 42: 123–130.

- DREW, G. M. & WHITING, S. B. 1979. Evidence for two distinct types of postsynaptic α -adrenoceptor in vascular smooth muscle in vivo. *Br J Pharmacol* 67: 207–215.
- EDVINSSON, L. & MACKENZIE, E. T. 1977. Amine mechanisms in the cerebral circulation. *Pharmacol Rev* 28: 274–348.
- EDVINSSON, L. & OWMAN, C. 1974. Pharmacological characterization of adrenergic α - and β -receptors mediating the vasomotor responses of cerebral arteries in vitro. *Circ Res* 35: 835–849.
- FURCHGOTT, R. F. 1972. Classification of adrenoceptors (adrenergic receptors): An evaluation from the standpoint of receptor theory. *In* Handbook of experimental pharmacology, vol. 33 (ed. H. Blaschko & E. Muscoll), pp. 283–355. Springer-Verlag, New York.
- HOFFMAN, B. B., LAVIN, T. N., LEFKOWITZ, R. J. & RUFFOLO, R. R. 1981. Alpha-adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. *J Pharmacol Exp Ther* 219: 290–295.
- HÖGESTÄTT, E., ANDERSSON, K.-E. & EDVINSSON, L. 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. 117: 49–61.
- KAPUR, H. & MOTTRAM, D. R. 1978. A comparative study on the pre- and postsynaptic alpha blocking activity of a series of benzodioxanes. *Biochem Pharmacol* 27: 1879–1880.
- LANGER, S. Z. 1974. Presynaptic regulation of catecholamine release. *Biochem Pharmacol* 23: 1793–1800.
- LANGER, S. Z. & SHEPPERSON, N. B. 1981. Subclassification of α -adrenoceptor into α_1 - and α_2 -categories: relevance to antihypertensive therapy. New trends in arterial hypertension. *In* Symposium No 17 (ed. M. Worel et al.). Elsevier/North Holland Biochemical Press.
- LANGER, S. Z., MASSINGHAM, R. & SHEPPERSON, N. B. 1980. Presence of postsynaptic α_2 -adrenoceptors of predominantly extrasynaptic localization in the vascular smooth muscle of the dog hind limb. *Clin Sci* 59: 225–228.
- PATEL, P., BOSCE, D. & GREENWAY, C. 1981. Effects of prazosin and phenoxybenzamine and α - and β -receptor-mediated responses in intestinal resistance and capacitance vessels. *J Cardiovasc Pharm* 3: 1054–1059.
- SKÄRBY, T., ANDERSSON, K.-E. & EDVINSSON, L. 1981. Characterization of the postsynaptic α -adrenoceptor in isolated feline cerebral arteries. *Acta Physiol Scand* 112: 105–107.
- STARKE, K. 1981. α -Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol* 88: 199–236.
- TIMMERMANS, P. B. M. W. M. & VAN ZWIETEN, P. A. 1981. The postsynaptic α_2 -adrenoceptor. *J Auton Pharmac* 1: 171–183.
- WEITZELL, R., TANAKA, T. & STARKE, K. 1979. Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn-Schmiedeberg's Arch Pharmacol* 308: 127–136.
- WEMER, R., VAN DER LUGT, J. C., DE LANGEN, C. D. & MULDER, A. H. 1979. On the capacity of presynaptic alpha receptors to modulate norepinephrine release from slices of rat neocortex and the affinity of some agonists and antagonists for these receptors. *J Pharmacol Exp Ther* 211, No. 3.
- WIKBERG, J. E. S. 1979. The pharmacological classification of adrenergic α_1 and α_2 receptors and their mechanisms of action. *Acta Physiol Scand* 68. Suppl. 468.



**CONTRACTION-MEDIATING α -ADRENOCEPTORS
IN ISOLATED HUMAN OMENTAL, TEMPORAL AND PIAL ARTERIES**

By

Tor Skärby and Karl-Erik Andersson

Running title: Human vascular α -adrenoceptors.

SUMMARY:

1. The α -adrenoceptors mediating contraction in human omental (OA), temporal (TA), and pial (PA) arteries obtained during surgery, were characterized by means of subtype selective agonists and antagonists.

2. In the OAs and TAs, prazosin concentration-dependently shifted the noradrenaline (NA) concentration-response (cr) curve towards higher concentrations, without depression of maximum. The corresponding Schild plots had slopes close to unity. Also rauwolscine caused a rightward displacement of the NA cr-curve in both OAs and TAs, without affecting the maximum response. In the TAs, rauwolscine in concentrations below 3×10^{-6} M appeared to be unable to shift the NA cr-curve. In the OAs, rauwolscine 3×10^{-8} M shifted the curve and the Schild plot seemed to be biphasic. Oxymetazoline, but not clonidine, produced contractile responses in the TAs and OAs, and phenylephrine was a full agonist in both types of vessel.

3. The PAs showed a pronounced inter- and intra-individual variation in the response to NA, and often exhibited spontaneous activity. Prazosin was considerably more effective than rauwolscine and yohimbine to inhibit NA-induced responses. Clonidine had no contractant effect, whereas oxymetazoline was more, and phenylephrine less potent than NA.

4. It is concluded that in human OAs, TAs and PAs, the α -adrenoceptor mediating contraction is mainly of α_1 -type.

Key words: Human vascular α -adrenoceptors - α_1 - and α_2 -adrenoceptors - omental arteries - temporal arteries - pial arteries - Schild plot

INTRODUCTION

Using drugs with selectivity for α_1 - and α_2 -adrenoceptors, it is possible to distinguish both of these subtypes postjunctionally in smooth muscle. Thus, phenylephrine stimulates preferentially α_1 -adrenoceptors, whereas the agonist clonidine has a certain selectivity for α_2 -adrenoceptors. Among antagonists, rauwolscine and yohimbine preferentially interact with α_2 -adrenoceptors, whereas prazosin blocks α_1 -adrenoceptors with a high degree of selectivity (for review see Langer & Shepperson 1981).

Most studies examining the pharmacological characteristics of vascular α -adrenoceptors, have been performed in animals. The α -adrenoceptor subtypes mediating contraction in vascular smooth muscle vary not only between different species (Ruffolo, Waddell & Yaden 1982), but also between different regions within the same species (Ruffolo, Waddell & Yaden 1981; Horn, Kohli, Listinsky & Goldberg 1982; Skärby, Andersson & Edvinsson 1983).

Information concerning the α -adrenoceptor subtype mediating contractions in different vascular regions in man may have not only theoretical interest, but also important clinical implications. Both α_1 - and α_2 -adrenoceptor stimulation has been shown to cause an elevation of blood pressure in man (Elliott & Reid 1983). Blockade of either of these subtypes with prazosin or yohimbine increased forearm blood flow (Amann, Bolli, Kiowski & Bühler 1980, van Brummelen, Vermey, Timmermans & van Zwieten 1982). Based on measurements of human nasal blood flow, Andersson & Bende (1984) suggested that resistance vessels in the nasal mucosa were endowed with α_2 -adrenoceptors, whereas α_1 -adrenoceptors prevailed in the capacitance vessels. In human isolated hand vessels, results have been obtained indicating that both α_1 - and α_2 -adrenoceptors may mediate contraction (Stevens & Moulds 1981; Flavahan & Mcgrath 1984). Whereas α_1 -adrenoceptors predominantly mediated contractions in isolated femoral and groin arteries, α_2 -adrenoceptors appeared to be the dominating subtype in the corresponding veins (Glusa & Markwardt 1983; Steen, Sjöberg, Skärby, Norgren & Andersson 1984).

The present study was performed on human arteries excised during surgery, to obtain information concerning the α -

adrenoceptors mediating contraction in omental, temporal and pial arteries.

MATERIAL AND METHODS

Omental arteries (OAs) with diameters of approximately 1 mm were removed from 18 patients (10 men, 8 women, age range 30-84 years) undergoing laparotomy for various intraabdominal disorders. Patients on drug therapy were excluded. Branches (diameter 2 mm) of temporal arteries (TA) were obtained from 12 patients (6 men, 6 women, age range 33-65 years) undergoing craniotomy in connection with operations for gliomas or ruptured intracranial aneurysms. The pial arteries (PA) included in the study were obtained from 15 patients (7 men, 8 women, age range 26-75 years). The pial vessels had a diameter of 1/2-1 mm and were removed during neurosurgical operations, in most cases performed in order to remove cerebral gliomas. After premedication with atropine and diazepam, operations were performed under general anaesthesia: sodium thiopenthal, suxamethonium, fentanyl, pancuronium and ventilation with a gas mixture of 60 % N₂O and 40 % O₂.

The specimens were immediately placed in a chilled Krebs buffer solution of the following composition (in mM): NaCl 119, NaHCO₃ 15, KCl 4.6, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2 and glucose 11. The buffer in the experiments with TAs and OAs also contained cocaine 10⁻⁶ M and propranolol 10⁻⁷ M for blockade of neuronal uptake and beta-adrenoceptors, respectively. Preliminary experiments indicated that higher concentrations of cocaine or propranolol did not increase the NA-induced responses in OAs and TAs, and neither cocaine nor propranolol appeared to augment the contractions elicited by NA in the PAs.

In the laboratory, the arteries were carefully dissected out from the adherent tissue. Part of the vessels was used immediately whereas the remainder was stored in a refrigerator for later use (within 24 hours). Segments (1 - 2 mm long) were suspended between two L-shaped metal holders immersed in tissue baths containing the same buffer solution. Care was taken to avoid any type of injury to the vessels. One metal holder was fixed to a Grass FT03C transducer, the output from which was amplified and recorded on a Grass polygraph. The other holder was attached to a movable unit allowing fine adjustments of vessel tension. In the baths, the buffer solution was continuously bubbled with 95 % O₂.

and 5 % CO₂, giving a pH of approximately 7.4. The temperature was kept at 37° C. During a period of 1/2 - 2 hours (depending on the type of vessel) the specimens were frequently stretched until a plateau of approximately 8 (TAs), 4 (OAs) and 3 (PAs) mN was reached.

All TAs and OAs and some PAs were thereafter contracted by a K⁺-rich buffer, in which the NaCl had been exchanged for KCl (giving 124 mM K⁺). In these vessels the contractions induced by agonists were expressed as a percentage of the K⁺-induced contractions in the same segment. When two reproducible contractions by K⁺ (variation < 10 %) had been elicited, nor-adrenaline (NA) was added cumulatively.

Because of the pronounced tachyphylaxis observed in some OAs, five or six segments from the same OA were examined in parallel, and only one cumulative concentration-response (cr) curve was constructed in each segment. Two segments were used as controls, receiving no drug but NA. Different agonists were added cumulatively to the other segments, or various concentrations of antagonist were administered to these three or four baths at least 15 min prior to the cumulative addition of NA.

In the TAs, two reproducible NA cr-curves were required before an antagonist or another agonist was added. The NA cr-curves were reproducible during several consecutive cumulative additions of the agonist. Therefore, the effects of three concentrations of antagonist (in increasing order) were examined in the same segment. The antagonists were introduced in the baths 15-20 min prior to each cumulative addition of NA.

All PAs included in the study (approximately 1/3 of the PAs examined), responded to NA (10⁻⁴ M) with two reproducible contractions exceeding 2 mN. It cannot be excluded that a certain tachyphylaxis occurred after these two control responses. Furthermore, in contrast to the OAs, the variation in response to NA between different segments from the same patient was pronounced. Some vessels responded well to K⁺ but were unable to contract upon exposure to NA. Due to the difficulties with these vessels, the effects of antagonists were mainly examined on contractions induced by a single NA contraction (10⁻⁵ M).

After two reproducible control contractions by NA (10^{-5} M), rauwolscine (10^{-8} M) or prazosin (10^{-10} M) was added to the baths at least 15 min prior to the next addition of NA. After washout of the NA-induced contraction the antagonist was added to the baths in a concentration ten times higher than the previous. The effects of increasing concentrations of antagonists were examined similarly. When the effects of other agonists were examined in the PAs, NA cr-curves were always obtained in the same segment.

In the experiments on PAs, the effects of a drug were mostly examined on two segments from the same patient, and the mean of these responses was included in the results.

The EC_{50} -value, i.e. the drug concentration at which half maximum effect is obtained, was determined graphically for each curve. The negative logarithm of the EC_{50} -value is denominated pEC_{50} and E_{max} denotes the maximum effect of a drug. PA_2 -values, i.e., the negative logarithm of the antagonist concentration causing a twofold shift of the agonist cr-curve towards higher concentrations, were determined using the method described by Arunlakshana and Schild (1959) (Schild plot) unless otherwise stated. Linear least square regressions of the Schild plots, were computed on a calculator (Hewlett Packard 41 CV). The program used for this calculation was kindly supplied by Dr Claus Rerup, Department of Pharmacology, University of Lund. The PA_2 -value of rauwolscine in temporal arteries was calculated according to the method described by van Rossum (1963): $PA_2 = pA_x + \log(CR-1)$ where pA_x is the negative logarithm of the antagonist concentration and CR is the concentration ratio, calculated as the ratio of the EC_{50} -values in absence and presence of antagonist. The NA cr-curves did not always reach a plateau when the highest concentrations of antagonists were present in the baths. In those cases, the EC_{50} -values were calculated assuming E_{max} to be unchanged.

Statistical significance was determined by paired or unpaired Student's t-test. A probability level of lower than 0.05 was accepted as significant. The values from the Schild plots are given as mean with 95 % confidence interval whereas the other result are given as mean $\pm$ SEM with n referring to the number of

vessels, each of which was excised from a different patient.

Drugs

Clonidine HCl (Boehringer Ingelheim), cocaine HCl (Merck), ($\pm$)-noradrenaline HCl, (-)-phenylephrine HCl, yohimbine HCl (Sigma), oxymetazoline chloride (Draco), prazosin HCl (Pfizer), propranolol HCl (ICI), rauwolscine HCl (Roth). Propranolol was supplied in ampoules. Prazosin (1 mM) was dissolved in 10 % lactic acid or 1 % methanol and 1 mM HCl. The other drugs were dissolved in 0.9 % NaCl and 1 mM ascorbic acid. Stock solutions of the drugs were stored at -70°C until use. Fresh dilutions in NaCl and ascorbic acid were prepared on the day of the experiment. Chemicals of analytical grade and double distilled water were used throughout.

RESULTS

The OAs were never seen to develop spontaneous contractions whereas a few of the TAs did. However, the PAs often exhibited spontaneous contractions. These contractions were often obtained as response to washing, but could also occur without provocation. In a few experiments it was observed that these spontaneous contractions could be abolished by low concentrations of nifedipine or by calcium free medium.

The amplitude of the K^+ -induced contraction was (in mN) 16.6 ± 2.4 , 29.4 ± 4.4 , and 9.0 ± 1.4 in the OAs ($n=18$), TAs ($n=12$), and PAs ($n=9$), respectively. The contractile responses elicited by 10^{-4} M NA were $104 \pm 4\%$, $111 \pm 3\%$ and $53 \pm 12\%$, respectively, when expressed as a percentage of the K^+ -induced responses. Fig 1 shows cr-curves obtained by cumulative addition of NA in OAs ($n=18$), TAs ($n=12$) and PAs ($n=11$). NA was significantly ($p < 0.05$) more potent in the TAs than in the OAs. When the results from all experiments were pooled, the NA pEC_{50} -values were 6.01 ± 0.08 , 6.28 ± 0.09 and 6.18 ± 0.10 in the OAs, TAs and PAs, respectively.

Omental arteries

Antagonists

Prazosin 10^{-9} - 10^{-7} M caused significant and concentration dependent shifts of the NA cr-curve towards higher concentrations, without depression of E_{max} (Fig 2a). The corresponding Schild plot resulted in a slope of unity and a pA_2 -value of 9.45 (see Table 1 and Fig 3).

Rauwolscine in the concentrations 3×10^{-8} - 10^{-5} M also significantly displaced the NA cr-curve to the right. The drug appeared to have a slight tendency to reduce the E_{max} . This tendency was, however, only significant with those antagonist concentrations in presence of which the NA cr-curves did not reach a well defined plateau (see Fig 2b). The Schild plot, illustrating the potency of rauwolscine, seemed to be biphasic (see Fig 3). Additional experiments performed in presence of 3×10^{-7} M propranolol, 3×10^{-5} M cocaine and 4×10^{-5} M corticosterone, did not shift the control NA cr-curve leftwards, or abolish the

biphasic appearance of the plot. If a regression line was drawn using the data obtained with the concentrations 10^{-8} , 3×10^{-8} and 10^{-7} M, the slope was 0.23, which differed significantly ($p < 0.05$) from unity. A regression line drawn in the concentration interval 10^{-6} - 10^{-5} M had a slope of 0.99. If a single regression line was calculated for the effects of all the concentrations 3×10^{-8} - 10^{-5} M, a slope of 0.67 was obtained which differed significantly ($P < 0.001$) from unity, and the locus of intersection with the abscissa was 7.27. Further information on the Schild plot, interpreted with a single regression line, is given in Table 1.

Agonists

Clonidine (10^{-8} - 10^{-4} M) was unable to elicit contractile responses in any of the arteries from 6 patients examined. Oxymetazoline contracted arteries from 4 of 6 patients. Whereas there was a considerable variation in the $E_{\max}$ for oxymetazoline in the arteries from these 4 patients (7-110 % of K^+), the pEC_{50} -value displayed little variation (7.26 - 7.66).

Both NA and phenylephrine were able to elicit concentration-dependent contractions in all the vessels examined. Whereas the maximum contractions caused by the two drugs were of similar magnitude, the pEC_{50} -value for NA was significantly higher than that for phenylephrine (Fig 4a, Table 2).

Temporal arteries

Antagonists

Prazosin (10^{-8} - 10^{-6} M) caused significant and concentration-dependent displacements of the NA cr-curves towards higher concentrations without depression of $E_{\max}$ (Fig 5a). The Schild plot (Fig 3), had a slope of 0.95, which did not differ significantly from unity, and the pA_2 -value was 9.21 (see Table 1).

Of the rauwolscine concentrations used (3×10^{-8} , 3×10^{-7} and 3×10^{-6} M), only 3×10^{-6} M was able to significantly shift the NA cr-curve to the right (Fig 5b). Using the shift produced by this concentration, the pA_2 -value, calculated according to van Rossum (1963), was 6.15 ± 0.13 ($n=5$).

Agonists

Clonidine caused no contractions in 3 vessels from 2 patients examined. However, oxymetazoline consistently produced concentration dependent contractions in all temporal arteries exposed to the drug (Fig 4b). Whereas the relative order of potency of agonists was: oxymetazoline > NA > phenylephrine the maximum contractile effects did not differ significantly between the three drugs (Table 2).

Pial arteries

Antagonists

In a few experiments (n=2) 10^{-8} M prazosin was found to be considerably more effective than 10^{-7} M yohimbine (Fig 6) in affecting the NA cr-curve. Prazosin appeared to produce a conspicuous reduction of E_{max} , and the drug was also substantially more potent than rauwolscine in inhibiting contractions induced by 10^{-5} M NA (see Fig 7 and 8).

Agonists

Clonidine (10^{-8} - 10^{-4}) was found to be without contractile effects in vessels from 3 patients examined. Oxymetazoline and phenylephrine consistently elicited concentration-dependent contractile responses in all the pial arteries exposed to the drugs (Fig 4c). The phenylephrine cr-curves did not always reach a plateau in the concentrations used (10^{-8} M - 3×10^{-4} M). For statistical comparison the pEC_{50} -value was estimated at the level of 50 % of the contraction obtained by 3×10^{-4} M phenylephrine. Despite this, phenylephrine was found to be approximately 20 times less potent than NA. Oxymetazoline, on the other hand, was about 300 times more potent than NA.

DISCUSSION

The amplitude of the contraction elicited by $NA\ 10^{-4}\ M$ was lower in cerebral than in peripheral arteries when expressed as a percentage of K^{+} -induced responses. This agrees well with previous studies on vessels from man, monkey, dog, (Toda 1983) cat (Skärby et al 1983) and rat (Högestätt & Andersson 1984). Furthermore, the pEC_{50} -values for NA in omental (6.01) and cerebral (6.18) arteries in the present study correlate well with those obtained in corresponding vessels from cat with the identical experimental set-up (6.04 and 6.19, respectively; Skärby et al. 1983). Thus, the potency of NA in these vascular regions seems to be similar in cat and man.

Omental arteries

The effects of prazosin on the NA cr-curve, showing an almost parallel displacement without reduction of E_{max} , and a slope of unity in the Schild plot, suggest a competitive interaction with one type of receptor (Arunlakshana & Schild 1959).

The pA_2 -value obtained, 9.45, agrees well with those found in functional studies on different vascular smooth muscle preparations, in which prazosin has been suggested to interact with postjunctional α -adrenoceptors of the α_1 -type. In these studies the pA_2 -values ranged between 7.9 and 9.9 (De Mey & Vanhoutte 1981; Caverio, Fenard, Gomeni, Lefevre & Roach 1978; Docherty & Starke 1981; Högestätt & Andersson 1984; Steen et al. 1984; Cohen, Wiley & Landry 1980). As the slope in the Schild plot was close to unity, the pA_2 -value should be a good estimate of the negative logarithm of the dissociation constant (K_D) (see Arunlakshana & Schild 1959; Kenakin 1982). The antilog of the $-pA_2$ value for prazosin in omental arteries (0.4 nM), corresponds well with K_D -values reflecting the interaction between prazosin and α_1 -adrenoceptors in several radioligand binding studies. These K_D -values ranged between 0.05 - 1.75 nM with a mean of 0.48 nM, as determined from 16 studies in various tissues (see Bylund & U'Prichard 1983). Furthermore, rauwolscine was less potent than prazosin in displacing the NA cr-curve towards higher concentrations, and phenylephrine, but not clonidine was able to elicit contractile responses. The potency ratio between NA and phenyl-

ephrine agrees well with that suggested to be characteristic for α_1 -adrenoceptors (Starke 1981). These results indicate that the α -adrenoceptors mediating contractions in the omental arteries are predominantly of the α_1 -type.

However, rauwolscine 3×10^{-8} M, which was suggested to interact selectively with α_2 -adrenoceptors in e.g., rabbit urethra (Andersson, Larsson & Sjögren 1984), caused a significant rightward shift of the NA cr-curve. The Schild plot, illustrating the antagonistic properties of rauwolscine, had an unusual appearance. It has been suggested that a competitive interaction with one type of receptor should yield a straight line with a slope of unity (Arunlakshana & Schild 1959; Kenakin 1982; Ruffolo 1982). The present data appear, however, to fit better with two lines; one with a slope significantly lower than unity in the concentrations 10^{-8} - 10^{-7} M and the other with a slope close to 1 in the concentrations 10^{-6} - 10^{-5} M. This appearance of the Schild plot is similar to that obtained when the uptake of NA is not sufficiently blocked (see Furchgott 1972; Kenakin 1982). However, the use of relatively high concentrations of cocaine and corticosterone did neither shift the control NA-cr-curve leftwards, nor did it abolish the biphasic appearance of the plot. Assuming the presence of a small population of α_2 -adrenoceptors, rauwolscine may have caused a blockade of these receptors in the concentrations 10^{-8} - 10^{-7} M. These concentrations can be regarded as interacting with α_2 -adrenoceptors with a relatively high degree of selectivity (Ferry, Goodhardt, Hanoune & Sevenet 1983; Andersson et al 1984). Kenakin (1982) stated: "if the agonist activates two receptors which mediate the same response, and the antagonist blocks only one of these, the slope of the Schild regression may be less than unity". Thus, it may be speculated that the slope in these concentrations, where it was significantly less than unity, may represent an interaction of rauwolscine with α_2 -adrenoceptors.

The pA_2 -values of rauwolscine, representing an interaction with α_1 -adrenoceptors, range between 5.3 and 5.9 (Weitzell, Tanaka & Starke 1979; Docherty & Starke 1981; Högestätt & Andersson 1984). The effects of the higher concentrations of rauwolscine (10^{-6} - 10^{-5} M) could therefore represent an interaction also with α_1 -

adrenoceptors. Consequently, the antagonist may in these concentrations behave as an unselective antagonist in the Schild plot, giving a slope close to unity. If the effects of rauwolscine were illustrated in the Schild plot, by a single regression line through all the data points, the slope was significantly lower than unity. This may, likewise, indicate that both α_1 - and α_2 -adrenoceptors mediate contractions in the preparation. Indeed, this interpretation agrees well with the suggestion by Flavahan & McGrath (1984). They proposed that yohimbine could produce a biphasic Schild plot when used in tissues containing both α_1 - and α_2 -adrenoceptors, and when regarded as linear, a slope of less than 1 would be obtained.

However, even if a contribution of α_2 -adrenoceptors cannot be excluded, the present results indicate that the contraction-mediating α -adrenoceptors in human omental arteries are mainly of the α_1 -type.

Temporal arteries

Prazosin 10^{-8} M - 10^{-6} M caused significant rightward shifts of the NA cr-curve without appreciable effects on $E_{\max}$. These findings together with a slope close to unity in the Schild plot indicate a competitive antagonism with one type of receptor. The pA_2 -value 9.21 correlates well with those suggested to represent an interaction of prazosin with α_1 -adrenoceptors (see above).

Rauwolscine was unable to produce a significant shift of the NA cr-curve in concentrations below 3×10^{-6} M. The pA_2 -value was therefore estimated according to van Rossum (1963). Although the pA_2 -value obtained, 6.15, can only be regarded as an estimation, it is considerably lower than the pA_2 -values reported for the interaction of this drug with α_2 -adrenoceptors in human femoral (7.48; Glusa & Markwardt 1983) and groin veins (9.03; Steen et al 1984) in functional studies. It is also lower than the negative logarithm of the k_i -value obtained in rabbit urethra with radioligand binding technique (8.10; Andersson et al 1984). It correlates better with pA_2 -values suggested to reflect the interaction of rauwolscine with α_1 -adrenoceptors (see above).

Furthermore, phenylephrine, but not clonidine, was able to elicit contractile responses, and the potency ratio between NA and phenylephrine corresponds well with that proposed to be representative for α_1 -adrenoceptors (Starke 1981). It may therefore be concluded that the contraction-mediating receptors stimulated by NA in these vessels are predominantly of α_1 -type.

Pial arteries

The interindividual variation of the NA-induced responses in pial arteries was pronounced, which agrees well with the results by Brandt, Ljunggren, Andersson & Hindfelt (1981). Because of the inconsistent responses in these vessels it is difficult to interpret the results obtained, on a strictly quantitative basis. However, the finding that phenylephrine but not clonidine was able to elicit contractions suggest a predominance of α_1 -adrenoceptors (Wikberg 1979). Despite this, oxymetazoline, which has been proposed to preferentially stimulate α_2 -adrenoceptors (Starke, Endo & Taube 1975, Starke 1981), was a potent agonist. However, previous results indicate that oxymetazoline may also potentially activate α_1 -adrenoceptors (Wikberg 1978). Furthermore, Högestätt & Andersson (1984) observed that this agonist concentration dependently contracted the rat basilar artery. This vessel has been suggested to be devoid of contraction-mediating α -adrenoceptors (Neild & Zelcer 1982); a finding which agrees well with the observation that NA, phenylephrine, and clonidine appeared to be unable to elicit contractile responses in this vessel (Högestätt & Andersson 1984). As these findings indicate that oxymetazoline in certain tissues may have contractile effects unrelated to α_2 -adrenoceptor stimulation, it may be questioned whether this drug could be a useful tool in the present system for α -adrenoceptor subclassification. This view is further supported by the results obtained using antagonists.

Yohimbine 10^{-7} M, which may be expected to antagonize α_2 -adrenoceptor mediated responses (Borowski, Starke, Ehrl & Endo 1977; Brodde, Hardung, Ebel & Bock 1982; Reichenbacher, Reimann & Starke 1982; Skärby et al 1983) appeared to be essentially devoid of effects on the NA cr-curves. On the other hand, prazosin seemed to have pronounced effects already in low concentrations and at a concentration of 10^{-8} M a conspicuous reduction of $E_{\max}$ was ob-

served. The effects of prazosin could, however, be washed out and the contractile responses to NA restored, indicating that the antagonist had a non-competitive, but not irreversible effect. Results indicating a non-competitive action of different α -adrenoceptor antagonists have previously been obtained in cerebral arteries from man, monkey (Toda 1983), dog (Sakakibara, Fujiwara & Muramatsu 1982; Toda 1983) and cat (Skärby et al 1983). The mechanism behind the non-competitive behaviour in cerebral vessels by antagonists, which cause a competitive inhibition in most peripheral arteries, is unclear (Toda 1983).

Although a certain tachyphylaxis could not be excluded, prazosin appeared to be substantially more potent than rauwolscine in inhibiting the contractions induced by NA (10^{-5} M). Even though the high potency of prazosin, to a certain extent could be explained by a non-competitive interaction of the drug, rauwolscine was considerably less potent in inhibiting NA-induced contractions in these vessels than in cat cerebral arteries (see Skärby 1984), in which NA mainly induces contractions via α_2 -adrenoceptors (Skärby, Andersson & Edvinsson 1981, 1983; Medgett & Langer 1983). Rauwolscine was also markedly less potent postjunctionally in the present study than prejunctionally in several other studies (e.g. Weitzell et al 1979; Docherty & Starke 1982; Skärby 1984). Thus, the present results suggest that the contraction-mediating α -adrenoceptors in human pial arteries are predominantly of α_1 -type.

The effects of prazosin and yohimbine on NA cr-curves have previously been examined in cerebral arteries from man (obtained during autopsy), monkey, and dog (Toda 1983). In good agreement with the present study cerebroarterial contractions induced by NA in man appeared to be predominantly mediated by α_1 -adrenoceptors. The same subtype was also suggested to prevail in monkey (Toda 1983) and rat cerebral arteries (Högestätt & Andersson 1984). On the other hand, α_2 -adrenoceptors seemed to be the main subtype in cat (Skärby et al 1981, 1983; Skärby 1984; Medgett & Langer 1983), dog (Sakakibara et al 1982; Toda 1983) and bovine (Tsukahara, Taniguchi, Fujiwara & Handa 1983) cerebral vessels. Thus, the type of α -adrenoceptor in cerebral vessels varies between different species.

In conclusion, the results of the present investigation suggest that the α -adrenoceptors mediating contraction in human omental, temporal, and pial arteries, are predominantly of α_1 -type.

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REFERENCES

- AMANN, F.W., BOLLI, P., KIOWSKI, W. & BÜHLER, F.R. (1980). Verstärkte α -Adrenoceptor-vermittelte Vasokonstriktion bei essentieller Hypertonie. *Schweiz Med Wochenschr* 110:1941-1944.
- ANDERSSON, K.-E. & BENDE, M. (1984). The role of adrenoceptors in the control of human nasal mucosal blood flow. *Ann Otol Rhin Laryngol* (In press).
- ANDERSSON, K.-E., LARSSON, B. & SJÖGREN, C. (1984). Characterization of the α -adrenoceptors in the female rabbit urethra. *Br J Pharmacol* 81:293-300.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br J Pharmacol* 14:48-59.
- BOROWSKI, E., STARKE, K., EHRL, H., & ENDO, T. (1977). A comparison of pre- and postsynaptic effects of α -adrenolytic drugs in the pulmonary artery of the rabbit. *Neurosci* 2:285-296.
- BRANDT, L., LJUNGGREN, B., ANDERSSON, K.-E. & HINDFELT, B. (1981). Individual variations in response of human cerebral arterioles to vasoactive substances, human plasma and CSF from patients with aneurysmal SAH. *J. Neurosurg.* 55:431-437.
- BRODDE, O.-E., HARDUNG, A., EBEL, H. & BOCK, K.D. (1982). GTP regulates binding of agonists to α_2 -adrenergic receptors in human platelets. *Arch Int Pharmacol* 258:193-207.
- BYLUND, D.B. & U'Prichard, D.C. (1983). Characterization of α_1 - and α_2 -adrenergic receptors. *International Review of Neurobiology*, Vol 24 (ed: Smythies & Bradley), pp 343-431. New York, Academic Press.
- CAVERO, I., FENARD, S., GOMENI, G., LEFEVRE, F. & ROACH, A.G. (1978). Studies on the mechanisms of the vasodilator effects of prazosin in dogs and rabbits. *Eur J Pharmacol* 49:259-270.
- COHEN, M.L., WILEY, K.S. & LANDRY, A.S. (1980). In vitro comparison of the pre- and postsynaptic alpha adrenergic receptor blocking properties of prazosin and tiodazosin (BL 5111). *Clin Exp Hypertens* 2:1067-1082.

- DE MEY, J. & VANHOUTTE, P.M. (1981). Uneven distribution of post-junctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 48:875-884.
- DOCHERTY, J.R. & STARKE, K. (1981). Postsynaptic α -adrenoceptor subtypes in rabbit blood vessels and rat anococcygeous muscle studied in vitro. *J Cardiovasc Pharmacol* 3:854-866.
- DOCHERTY, J.R. & STARKE, K. (1982). An examination of the pre- and postsynaptic α -adrenoceptors involved in neuroeffector transmission in rabbit aorta and portal vein. *Br J Pharmacol* 76:327-335.
- ELLIOTT, H.L. & REID, J.L. (1983). Evidence for postjunctional vascular α_2 -adrenoceptors in peripheral vascular regulation in man. *Clinical Science* 65:237-241.
- FLAVAHAN, N.A., McGRATH, J.C. (1984). Are human α -adrenoceptors atypical? *J Cardiovasc Pharmacol* 6:208-210.
- FERRY, N., GOODHARDT, M., HANOUNE, J. & SEVENET, T. (1983). Binding of yohimbine stereoisomers to α -adrenoceptors in rat liver and human platelets. *Br J Pharmacol* 78:359-364.
- FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: *Handbook of experimental pharmacology*, vol 33 (ed H. Blaschko & E. Muscoll), pp 283-355. Springer Verlag, New York.
- GLUSA, E. & MARKWARDT, F. (1983). Characterization of postjunctional α -adrenoceptors in isolated human femoral veins and arteries. *Naunyn-Schmiedeberg's Arch Pharmacol* 323:101-105.
- HORN, P.T., KOHLI, J.D., LISTINSKY, J.J. & GOLDBERG, L.I. (1982). Regional variation in alpha adrenergic receptors in the canine resistance vessels. *Naunyn-Schmiedeberg's Arch Pharmacol* 318:166-172.
- HÖGESTÄTT, E.D. & ANDERSSON, K.-E. (1984). On the postjunctional α -adrenoceptors in rat cerebral and mesenteric arteries. Submitted for publication.

- KENAKIN, T.P. (1982). The Schild regression in the process of receptor classification. *Can J Physiol Pharmacol* 60:249-265
- LANGER, S.Z. & SHEPPERSON, N.B. (1981). Subclassification of α -adrenoceptors into α_1 - and α_2 -categories: relevance to antihypertensive therapy. In: *New trends in arterial hypertension. Intern Symposium No 17* (ed M. Worcel et. al.) pp 73-85. Elsevier/North Holland Biomedical Press.
- MEDGETT, I.C. & LANGER, S.Z. (1983). Characterization of smooth muscle α -adrenoceptors and of responses to electrical stimulation in the cat isolated perfused middle cerebral artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 323:24-32
- NEILD, T.O. & ZELCER, E. (1982). Noradrenergic neuromuscular transmission with special reference to arterial smooth muscle. *Progress in Neurobiology* 19:141-158.
- REICHENBACHER, D., REIMANN, W., & STARKE, K. (1982). α -adrenoceptor-mediated inhibition of noradrenaline release in rabbit brain cortex slices. Receptor properties and role of the biophase concentration of noradrenaline. *Naunyn-Schmiedeberg's Arch Pharmacol* 319:71-77.
- RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. (1981). Postsynaptic alpha-adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of α_2 -adrenergic receptors in rat aorta. *J Pharmacol Exp Ther* 217:235-240.
- RUFFOLO, R.R. (1982). Important concepts of receptor theory. *J Auton Pharmac* 2:277-295
- RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. (1982). Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *J Pharmacol Exp Ther* 221:309-314.
- SAKAKIBARA, Y., FUJIWARA, M. & MURAMATSU, I. (1982). Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. *Naunyn Schmiedeberg's Arch Pharmacol* 319:1-7.
- SKÄRBY, T., ANDERSSON, K.-E. & EDVINSSON, L. (1981). Characterization of the postsynaptic α -adrenoceptor in isolated feline cerebral arteries 112:105-107.

- SKÄRBY, T.V.C., ANDERSSON, K.-E. & EDVINSSON, L. (1983). Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand* 117:63-73.
- SKÄRBY, T. (1984). Pharmacological properties of prejunctional α -adrenoceptors in isolated feline middle cerebral arteries; comparison with the postjunctional α -adrenoceptors. *Acta Physiol Scand* (In press)
- STARKE, K., ENDO, T., TAUBE, H.D. (1975). Relative pre and postsynaptic potencies of α -adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 291:55-78.
- STARKE, K. (1981). α -Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol* 88:199-236.
- STEEN, S., SJÖBERG, T., SKÄRBY, T.V.C., NORGREN, L. & ANDERSSON, K.-E. (1984). Postjunctional α_1 - and α_2 -adrenoceptors mediating contraction in isolated human groin arteries and veins. *Acta physiol Scand*. Accepted for publication.
- STEVENS, M.J. & MOULDS, R.F.W. (1981). Heterogeneity of post-junctional α -adrenoceptors in human vascular smooth muscle. *Arch Int Pharmacodyn* 254:43-57.
- TODA, N. (1983). Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. *J Pharmacol Exp Ther*. 226:861-868.
- TSUKAHARA, T., TANIGUCHI, T., FUJIWARA, M & HANDA, H. (1983). Characterization of alpha adrenoceptors in pial arteries from the bovine brain. *Naunyn-Schmiedeberg's Arch Pharmacol* 324:88-93.
- VAN BRUMMELEN, P., VERMEY, P., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1982). Preliminary evidence for a postsynaptic α_2 -adrenoceptor in the vasculature of the human forearm. *Proceedings of the B.P.S., 8th-10th September 1982*, 134P-135P.

- VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch Int Pharmacodyn 143:299-330
- WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. Naunyn-Schmiedeberg's Arch Pharmacol 308:127-136.
- WIKBERG, J. (1978). Differentiation between pre- and post-junctional α -receptors in guinea pig ileum and rabbit aorta. Acta Physiol Scand 103:225-239.
- WIKBERG, J.E.S. (1979). The pharmacological classification of adrenergic α_1 and α_2 receptors and their mechanism of action. Acta Physiol Scand Suppl 468.

Table 1. Effects of antagonists on the NA concentration-response curve in human peripheral arteries.

Artery	Antagonist	z	Slope of regression line	Correlation coefficient	pA ₂ -value
Omental	Prazosin	24	1.08 (0.92 - 1.24)	0.95	9.45 (9.23 - 9.74)
Omental	Rauwolscine	47	0.67 (0.49 - 0.86)	0.74	7.27 (6.96 - 7.74)
Temporal	Prazosin	18	0.95 (0.76 - 1.14)	0.93	9.21 (8.86 - 9.73)
Temporal	Rauwolscine ^x	5			6.15 (5.82 - 6.48)

Data calculated according to Arunlakshana & Schild (1959) except the pA₂-values for rauwolscine in temporal arteries (x) which were calculated according to van Rossum (1963). Values within brackets describe the 95 % confidence intervals, and z denotes the number of determinations.

Table 2. Contractile responses elicited by agonists in isolated human arteries.

A r t e r y	A g o n i s t			
	Clonidine	Oxymetazoline	NA	Phenylephrine
<u>Omental</u>		(n=4)	(n=8)	(n=6)
pEC ₅₀	No effect	7.44 ± 0.09	6.14 ± 0.14	5.63 ± 0.19
E _{max} (% of K ⁺)		13 ± 8	112 ± 10	106 ± 11
<u>Temporal</u>		(n=4)	(n=7)	(n=6)
pEC ₅₀	No effect	7.52 ± 0.13	6.40 ± 0.16	6.04 ± 0.07
E _{max} (% of K ⁺)		69 ± 19	109 ± 3	98 ± 6
<u>Pial</u>		(n=5)	(n=6)	(n=3)
pEC ₅₀	No effect	8.30 ± 0.09	6.22 ± 0.16	≤ 4.9
E _{max} (% of NA 10 ⁻⁴ M)		77 ± 18	100 ± 0	≥ 75

Each value represents mean ± SE and n denotes the number of patients with responding vessels.

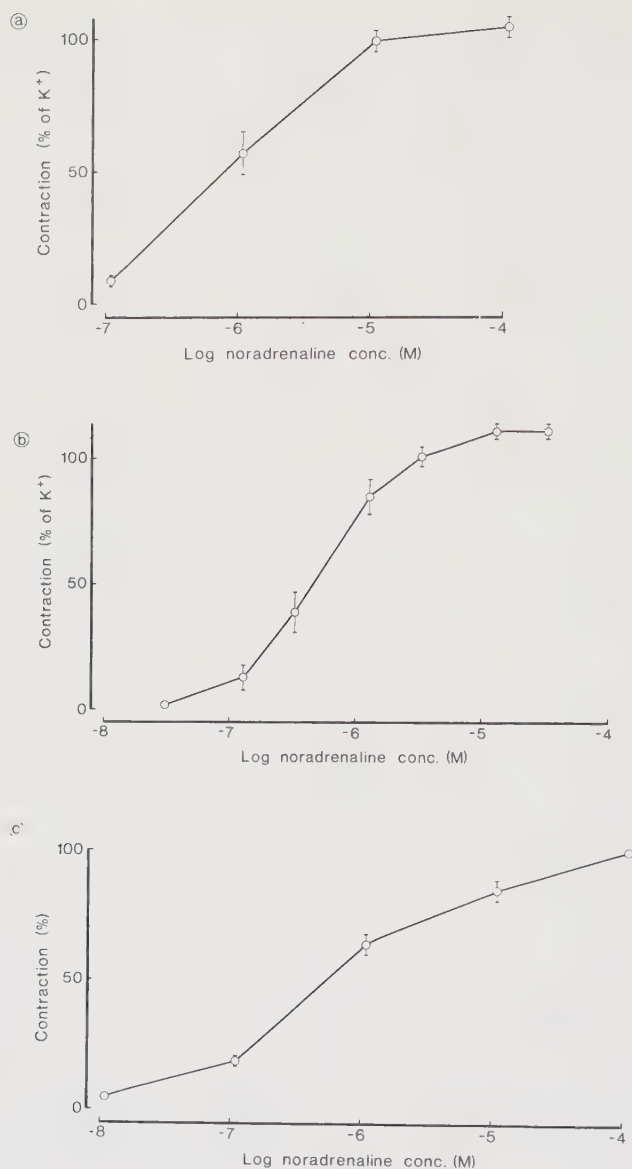


Fig 1 Concentration-response curves obtained by cumulative addition of noradrenaline to a) omental (n=18), b) temporal (n=12) and c) pial (n=11) arteries. The values obtained in omental and temporal arteries are expressed as a percentage of the K⁺-induced contraction, whereas the effects in pial arteries are expressed as a percentage of the contraction elicited by 10⁻⁴ M noradrenaline. Each value represents mean $\pm$ SEM.

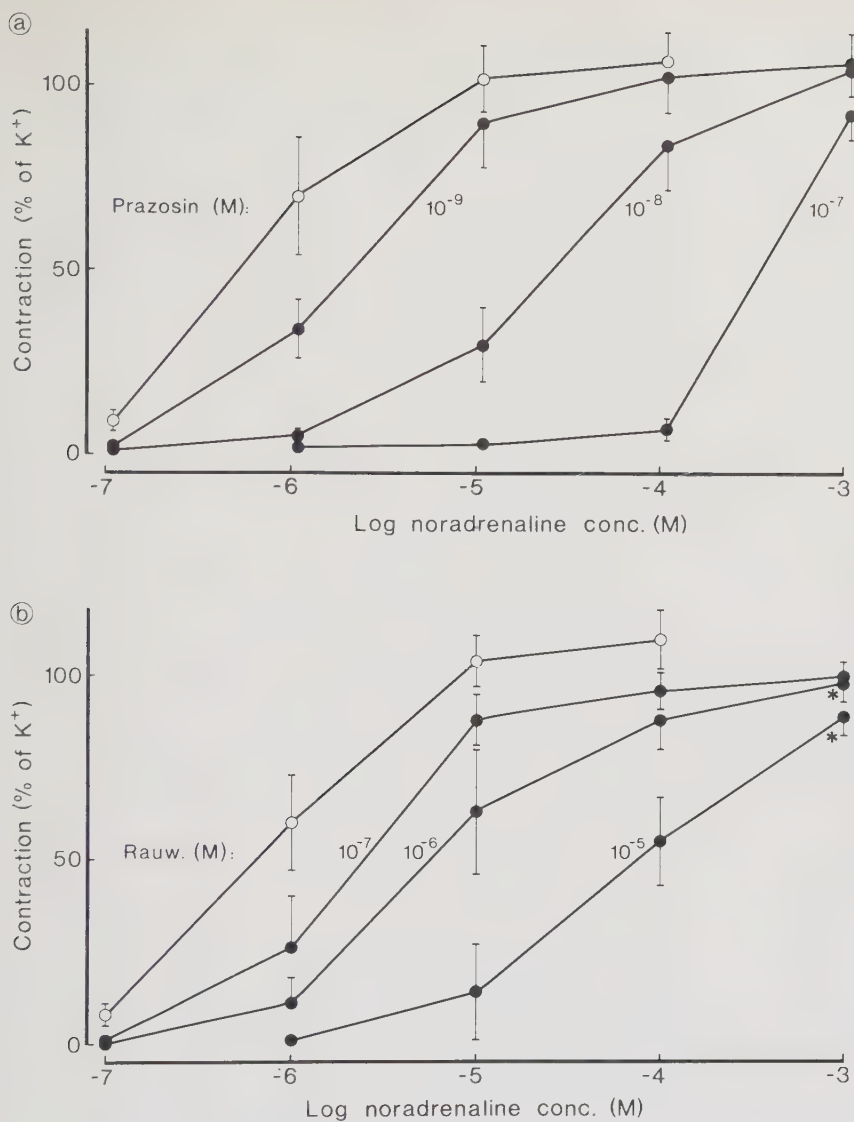


fig 2 Concentration-response curves obtained by cumulative addition of noradrenaline to omental arteries, in the absence (○) and presence (●) of antagonists : a) prazosin ; b) rauwolscine (Rauw). For clarity, only the effects of 10⁻⁷, 10⁻⁶ and 10⁻⁵ M rauwolscine are shown. Each value is expressed as a percentage of the K⁺-induced contraction and represents mean ± SEM (n=8). Asterisk denotes significant (P < 0.05) difference as compared to control.

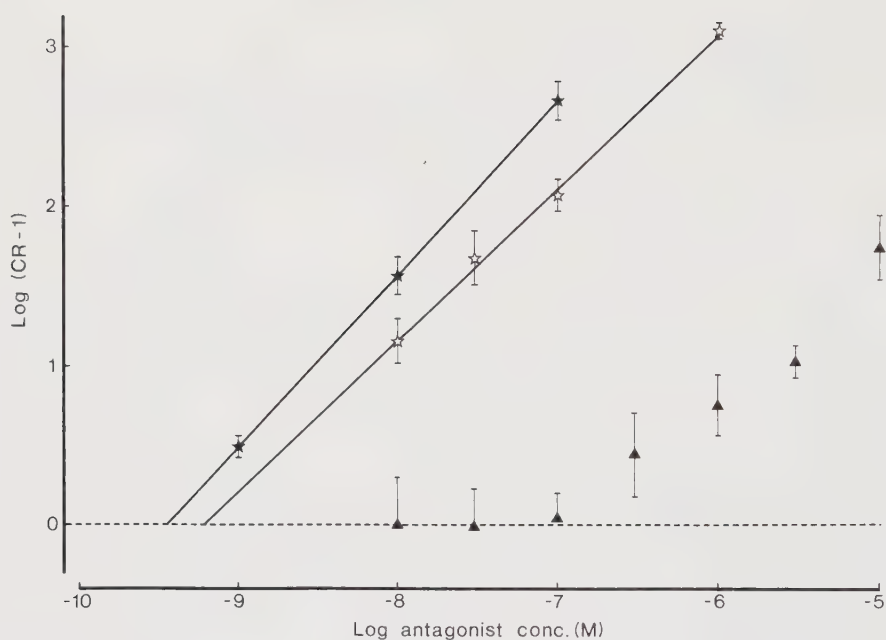


Fig 3 Schild plots illustrating the interaction between noradrenaline and prazosin in human omental (n=7-8; ★) and temporal (n=3-6; ☆) arteries; effects of rauwolscine in omental arteries (n=5-8; ▲) Each point represents mean $\pm$ SEM.

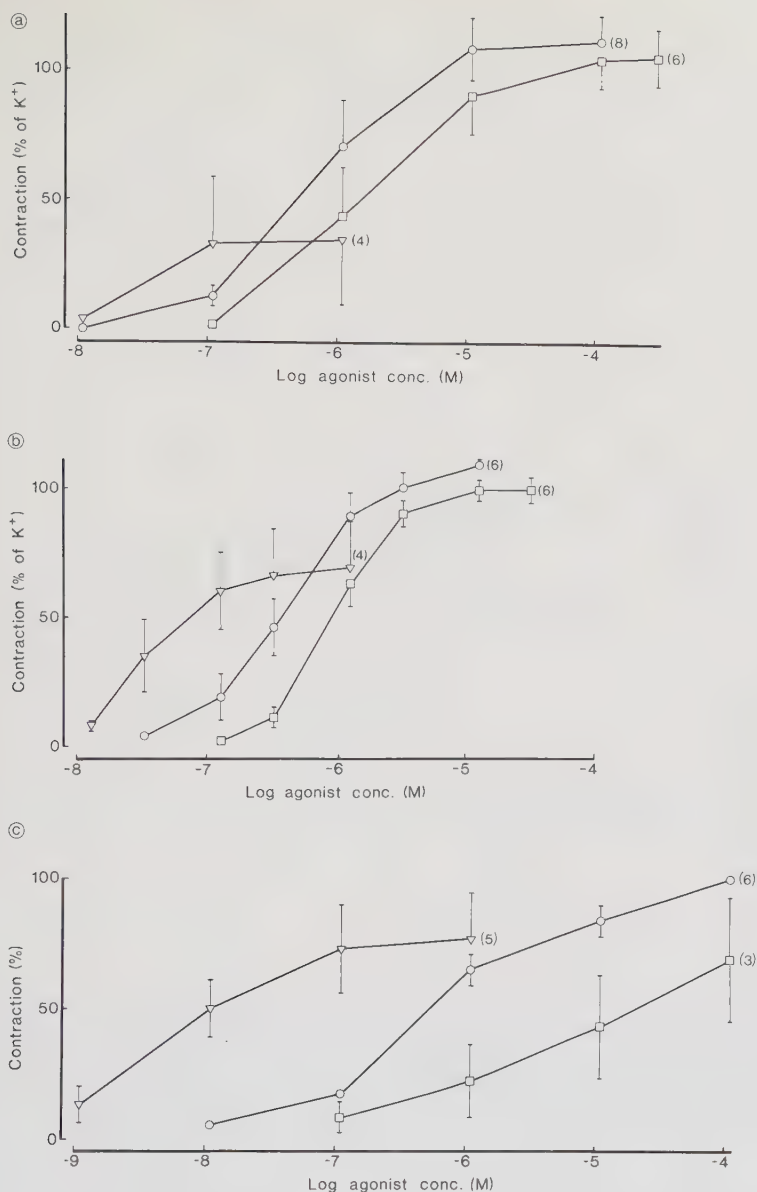


Fig 4 Concentration-response curves to noradrenaline (O), oxy-metazoline (∇) and phenylephrine (□) in a) omental b) temporal and c) pial arteries. Values represent mean $\pm$ SEM. In omental and temporal arteries they are expressed as a percentage of the K^+ -induced contractions. Responses in pial vessels are expressed as a percentage of the contractions induced by NA 10^{-4} M. Values within brackets represent number of experiments.

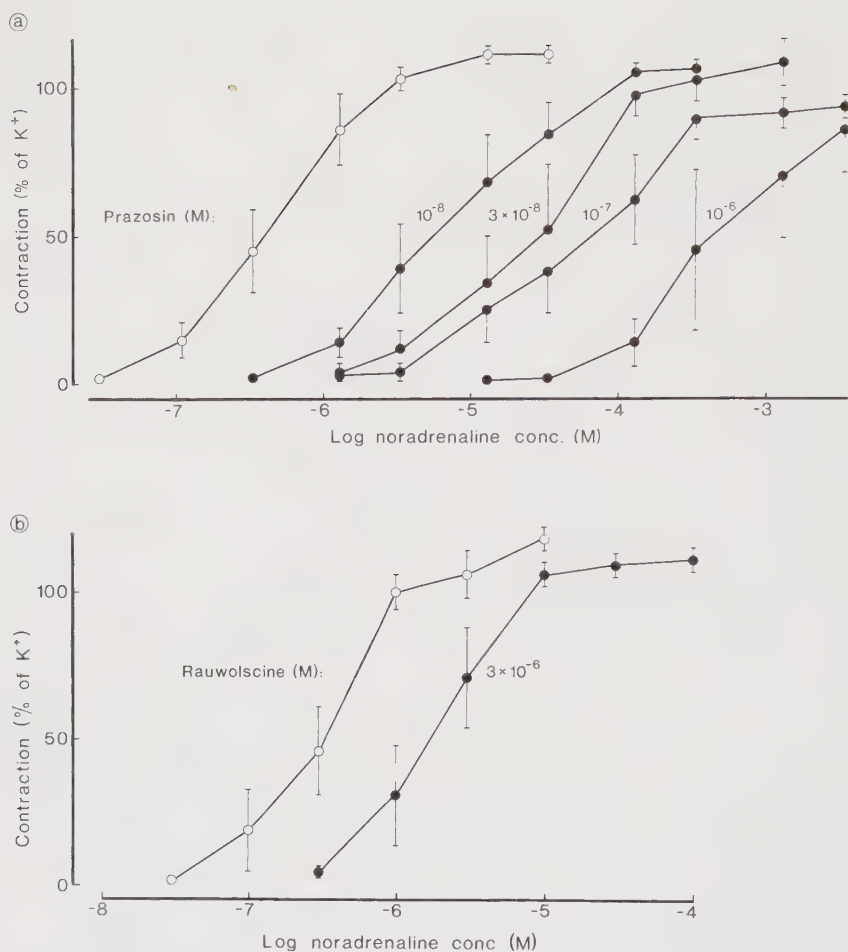


Fig 5 Concentration-response curves obtained by cumulative addition of noradrenaline to temporal arteries in the absence (○) and presence (●) of antagonist : a) prazosin; b) rauwolscine. Each point is expressed as a percentage of the K⁺-induced contraction and represents mean ±SEM of 5-7 determinations. The effects of prazosin 3 × 10⁻⁸ and 10⁻⁶ M were, however, only examined in 3 experiments.

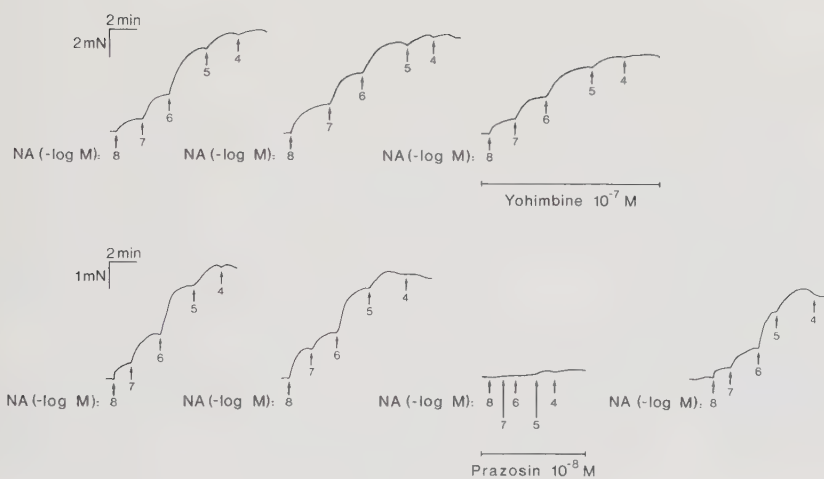


Fig 6 Tracings from an experiment illustrating the effects of yohimbine (10^{-7} M) and prazosin (10^{-8} M) on the contractile responses elicited by cumulative addition of noradrenaline (NA) in pial arteries. The antagonist was present at least 15 min prior to the addition of NA.

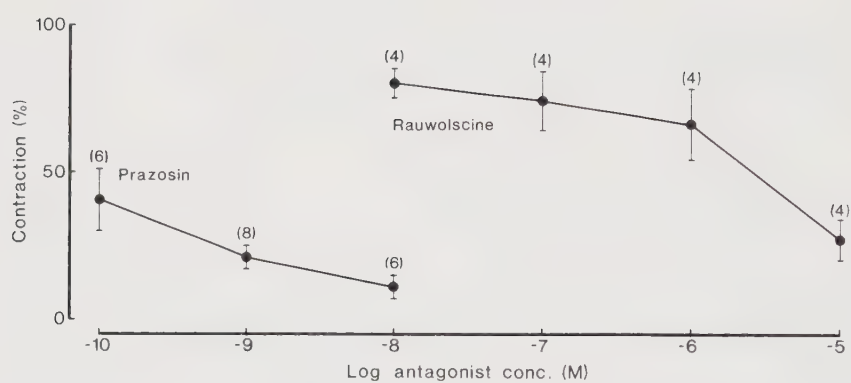


Fig 7 Effects of prazosin and rauwolscine on contractions induced by 10^{-5} M noradrenaline in pial arteries. Each value represents mean $\pm$ SEM. Figures within brackets denote number of experiments.

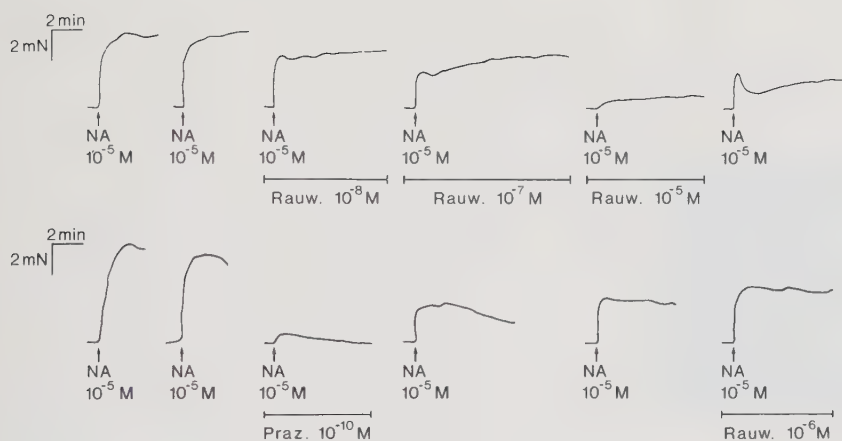
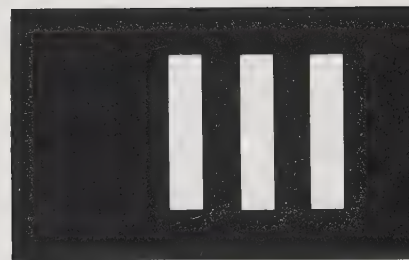


Fig 8 Tracings from an experiment illustrating the effects of rauwolscine (Rauw.) and prazosin (Praz.) on contractions elicited by 10^{-5} M noradrenaline (NA) in pial arteries. Antagonists were present in the baths at least 15 min prior to addition of NA.



PHARMACOLOGICAL PROPERTIES OF PREJUNCTIONAL α -
ADRENOCEPTORS IN ISOLATED FELINE MIDDLE CEREBRAL
ARTERIES; COMPARISON WITH THE POSTJUNCTIONAL
 α -ADRENOCEPTORS

by

Tor Skärby

Running title: α_2 -adrenoceptors in cat cerebral arteries

ABSTRACT

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In cat middle cerebral arteries (CMCA) preincubated with ^3H -noradrenaline (NA), the outflow of tritium evoked by electrical stimulation was reduced to 32 % by α -adrenoceptor (α -receptor) stimulation with oxymetazoline, and increased to 487 % by α -receptor blockade with HEAT. The relative order of potency for α -receptor agonists on prejunctional receptors was: clonidine $\geq$ oxymetazoline $>$ phenylephrine, and the antagonist rauwolscine was more potent than prazosin. This indicates that the prejunctional α -receptors are mainly of α_2 -type. Rauwolscine was more potent than prazosin in inhibiting the contractions induced by NA, indicating a predominance of α_2 -receptors postjunctionally. Apart from clonidine having higher intrinsic activity pre- than post-junctionally, all drugs examined (oxymetazoline, phenylephrine, rauwolscine, HEAT (BE2254), and prazosin) had similar concentration-effect curves on the pre- and postjunctional receptors. Furthermore, the ratios of EC_{50} -values pre- and post-junctionally of rauwolscine, oxymetazoline, and clonidine were all close to unity. These results indicate that pre- and post-junctional α_2 -receptors in the CMCA have similar pharmacological characteristics and can not be influenced separately by the presently used drugs.

Key words. Pre- and postjunctional α_2 -adrenoceptors, cat cerebral arteries, ^3H -noradrenaline release, isometric contractions.

INTRODUCTION

Postjunctional α -receptors of both α_1 - and α_2 -type have been suggested to mediate contraction in vascular smooth muscle. The occurrence of these subtypes varies between different vascular regions (Davey 1980, De Mey & Vanhoutte 1981, Ruffolo et al 1981, Horn 1982, Skärby et al 1983) and species (Ruffolo et al 1982, Skärby et al 1983, Toda 1983, Högestätt & Andersson 1984). On the other hand, much information indicates that the prejunctional α -receptors in most tissues investigated are of the α_2 -type (cf Langer 1981).

Recent studies on isolated vessels in man indicate the presence of postjunctional α_2 -receptors in digital arteries and metacarpal veins (Stevens & Moulds 1982), and in omental (Steen et al 1984a), femoral (Glusa & Markwardt 1983), and groin veins (Steen et al 1984b). In vivo, postjunctional α_2 -receptors have been suggested to mediate a blood pressure increase (Elliott & Reid 1983) and to influence forearm blood flow (van Brummelen et al 1982). If pre- and postjunctional α_2 -receptors have the same pharmacological properties, an α_2 -receptor antagonist administered in order to reduce α_2 -receptor mediated vasoconstriction would block also prejunctional α_2 -receptors. Thereby an increased noradrenaline outflow from sympathetic nerves, causing unwanted effects, could be expected.

The present nomenclature implies that pre- and postjunctional α_2 -receptors do not differ in sensitivity to α -receptor agonists and antagonists. However, Hicks (1981) using the α -receptor antagonist HEAT (BE2254), obtained results indicating a difference between these two types of α_2 -receptor. Therefore, further investigations seem necessary to establish whether or not differences can be demonstrated between pre- and postjunctional α_2 -receptors.

The present study was performed to compare the pharmacological characteristics of pre- and postjunctional α_2 -receptors in the cat middle cerebral artery (CMCA), which previously has been shown to be predominantly endowed with α_2 -receptors postjunctionally (Skärby et al 1981, 1983, Langer & Medgett 1982, Medgett & Langer 1983).

MATERIAL AND METHODS

Cats of either sex were anaesthetized with pentobarbital (Mebumal^R, 30 mg/kg) and sacrificed by injection of air (approximately 10 ml) into the heart. After decapitation, the brain was immediately removed and immersed in cold buffer solution (for composition, see below).

Isotope experiments.

Under a dissecting microscope the middle cerebral arteries were cut at the origin of the circle of Willis and the vessels and their branches were carefully removed from the brain surface. All vessels on the brain surface in the spreading area of the CMCA, together with adherent pia mater, were included. Part of the tissue was immediately incubated at 37° C in buffer solution (for composition, see below) containing 0.3 μ M ³H-L-noradrenaline (specific activity 47.7 Ci/mmol) for 45 min, whereas the rest was stored in a refrigerator for up to 7 hours for use in subsequent experiments. After incubation, the tissue was rinsed 3 x 10 min in different vials, each containing 10 ml buffer solution at room temperature, and thereafter distributed between metal nets in temperature controlled perspex-chambers, each with a volume of 0.3 ml. A buffer solution of the following composition (mM): NaCl 119, NaHCO₃ 15, KCl 4.6, NaH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 1.5, glucose 11, was perfused through the chambers with a flow rate of 3.8 ml/min. The buffer solution was kept at 37° C and was bubbled with a mixture of 95 % O₂ and 5 % CO₂, giving a pH of approximately 7.4.

After 120 minutes the buffer was changed to one containing in addition (M): ascorbic acid 3×10^{-4} , Na₂ EDTA 3×10^{-5} , propranolol 4×10^{-7} , cocaine 3×10^{-5} , and corticosterone 4×10^{-5} . After another 15 minutes the flow rate was reduced to 1.8 ml/min, and fraction collection into scintillation vials (3 min/fraction) was started 150 minutes after onset of perfusion.

Alternating square wave pulses of 2 Hz, 0.3 ms duration and 150 mA current strength (Grass S48) were delivered over 150 sec, beginning at 168 (S₁), 189 (S₂), 210 (S₃), 231 (S₄), 252 (S₅), and 273 (S₆) minutes after onset of perfusion.

At the end of the experiment the tissue was dissolved in 2 ml of Soluene^R (Packard) or Lumasolve^R (Lumac), and 10 ml Lumagel SB^R (Lumac) was added to each vial. The radioactivity was determined by means of a liquid scintillation spectrometer (LKB, Rack-Beta 1215). Counting efficiency was approximately 20 % as determined with the external standard channels ratio method. For evaluation, the radioactivity of each sample was calculated as percentage of remaining tissue radioactivity (fractional release). Stimulation Evoked Fractional Release (SEFR) was calculated as the fractional release of ³H measured during the period of stimulation, plus release in the following two collection periods. The estimated basal overflow was subtracted, assuming it to be constant during these 9 minutes and similar to the collection period of 3 minutes preceding the stimulation (see Fig 1). The first SEFR (S₁) served as control and the subsequent SEFRs (S_n) were expressed as S_n/S₁.

Clonidine, oxymetazoline and phenylephrine were added in increasing concentrations 12 minutes before each stimulation period. Thus, 10⁻⁹ M, 10⁻⁸ M, 10⁻⁷ M, 10⁻⁶ M, and 10⁻⁵ M of the drugs were present during the 2nd (S₂), 3rd (S₃), 4th (S₄), 5th (S₅), and 6th (S₆) stimulation period, respectively. In preliminary experiments it was observed that the increase in SEFR obtained by rauwolscine 10⁻⁷ M, appeared to be lower if the effects of this concentration were examined during S₄, preceded by S₂ and S₃ in the presence of rauwolscine 10⁻⁹ and 10⁻⁸ M, than if the effects by 10⁻⁷ M were investigated during S₂. This was probably due to the massive augmentation in SEFR caused by 10⁻⁹ and 10⁻⁸ M rauwolscine which may have attenuated the amount of NA available in the subsequent stimulation. Therefore, the effects by 10⁻⁹ and 10⁻⁸ M of the antagonists were examined during S₂ and S₃ respectively, whereas 10⁻⁷ and 10⁻⁶ M rauwolscine, HEAT, and prazosin, and 10⁻⁵ M rauwolscine were in separate experiments added 12 min before S₂. Drug effects described in results, discussion, and figures were evaluated by expressing the ratio S_n/S₁ as percentage of the corresponding S_n/S₁-ratio in control experiments. Interactions of the agonists clonidine or phenylephrine with the antagonists rauwolscine or prazosin were examined by adding the agonists as previously described while the perfusion medium contained 10⁻⁹ M

rauwolscine or 10^{-7} M prazosin during the whole experiment (S_1 - S_6).

Concentration-contractile response curves.

Segments of the CMCA were mounted on two L-shaped stainless steel wires (0.2 mm in diameter), one of which was attached to a Grass FT03 transducer and a Grass polygraph for continuous recording of "isometric" tension. The other holder was fixed to a movable unit for fine adjustment of vessel tonus (for further experimental details, see Högestätt et al 1983). Each temperature-controlled tissue bath contained 5 ml of buffer solution with the same ionic composition as described previously, and propranolol 3×10^{-7} M was present to block the β -adrenoceptors. In the baths the buffer solution was bubbled with a mixture of 5 % CO_2 and 95 % O_2 , giving a pH of approximately 7.4. After mounting, the vessels were subjected to an initial tonus of 5 mN resulting in an immediate relaxation. To compensate for this the preparation was frequently stretched. Approximately 45 min later, when a steady tension level of 4 mN had been reached, noradrenaline (NA) 10^{-5} M was added to the vessel baths. Previous studies (Skärby et al 1983) have shown that 10^{-5} M of NA caused a contraction which was approximately 95 % of the maximum NA-induced contraction. When two reproducible contractions of NA 10^{-5} M had been obtained, an antagonist was added in its lowest concentration. At least 15 min later, the vessels were again contracted with 10^{-5} M NA. After thorough washing, another contraction by NA was elicited in the presence of an antagonist concentration 10 times higher than before. This procedure was repeated until maximum inhibition of the NA contraction was achieved. In control experiments it was observed that the NA-induced contractions remained reproducible during the course of the experiments.

The E_{max} -value is defined as the maximum effect obtained with a drug (increase or decrease), and the pEC_{50} -value is defined as the negative logarithm of the EC_{50} -value, which is the drug concentration at which 50 % of maximum effect occurs.

Student's t-test was used to determine statistical significance. The 0.05 level of probability was accepted as significant. The results are given as mean $\pm$ standard error.

Drugs

(Ring-2,5,6-³H)-L-Norepinephrine (New England Nuclear), clonidine HCl (Boehringer Ingelheim), cocaine HCl (Merck), corticosterone, L-phenylephrine HCl (Sigma), oxymetazoline chloride (Draco), propranolol HCl (ICI), prazosin HCl (Pfizer), rauwolscine HCl (Roth), HEAT (BE 2254) (2-(β -(4-hydroxyphenyl)-ethyl-aminomethyl)-tetralone) (Beirsdorf, A.G.), and tetrodotoxin (Sigma). Drugs were dissolved in 0.9 % NaCl containing 0.1 M ascorbic acid. Prazosin (1 mM) was dissolved in 10 % lactic acid or 2.5 mM in 1 % methanol and 1 mM HCl. Corticosterone was dissolved in ethanol prior to addition to the perfusion medium giving a final ethanol concentration of 0.1 % in the buffer. All drugs except propranolol and corticosterone were prepared and stored as stock solutions at -70° C. Fresh dilutions in NaCl and ascorbic acid were prepared on the day of the experiments. Redistilled water and chemicals of analytical grade were used throughout.

RESULTS

Isotope experiments

Controls

Control experiments showed the SEFR to vary slightly during the six consecutive stimulation periods (see Fig 1a and Table 1). When tetrodotoxin (TTX) 10^{-7} M was present or when calcium was omitted and exchanged for EGTA (10^{-5} M) in the perfusion buffer the SEFR was abolished.

Agonists

Clonidine decreased the SEFR in a concentration-dependent manner. A significant ($p < 0.05$) decrease was observed with 10^{-8} M clonidine and a maximum inhibition of $64 \pm 9\%$ ($n=5$) was reached at 10^{-5} M (Fig 1b and 2). With rauwolscine 10^{-9} M the pEC_{50} -value of the concentration-inhibition curve for clonidine was significantly ($p < 0.01$) shifted from 7.40 ± 0.15 ($n=5$) to 6.62 ± 0.11 ($n=5$) (see Fig 3). With prazosin 10^{-7} M in the perfusion medium the pEC_{50} -value for clonidine, 6.93 ± 0.16 ($n=5$), did not differ significantly from the value obtained in absence of the drug. However, prazosin 10^{-7} M attenuated significantly ($p < 0.05$) the effect caused by 10^{-8} M clonidine (The SEFR in presence of rauwolscine 10^{-9} M or prazosin 10^{-7} M was relatively unchanged during six consecutive stimulation periods, Table 1).

Oxymetazoline did also cause a significant ($P < 0.05$) decrease of the SEFR in the concentration 10^{-8} M, and the concentration-inhibition relationship was almost identical to that of clonidine (Fig 2). The pEC_{50} -value was 7.33 ± 0.08 with a maximum reduction of $68 \pm 6\%$ ($n=4$).

Phenylephrine caused a concentration-dependent decrease in SEFR with a pattern somewhat different from that of clonidine and oxymetazoline (Figs 1c and 2). A significant ($p < 0.05$) decrease occurred with the concentration 10^{-6} M, and a slight increase in basal efflux was observed with 10^{-5} M (Fig 1c). At this concentration the SEFR was estimated as the stimulation evoked increase in fractional release from the elevated basal efflux. Neither rauwolscine 10^{-9} M nor prazosin 10^{-7} M were able to significantly alter the effects of phenylephrine in any of the concentrations used.

Antagonists

Rauwolscine increased the SEFR concentration-dependently up to a concentration of 10^{-6} M. Already at a concentration of 10^{-9} M a significant ($p < 0.001$) enhancement was obtained. Maximum increase was observed at 10^{-6} M (Fig 2). At a concentration of 10^{-5} M SEFR was lower than maximum, but the difference was not significant.

Prazosin was without significant effects in the concentrations 10^{-9} and 10^{-8} M. However, prazosin 10^{-7} M enhanced SEFR significantly ($p < 0.001$) (Fig 2). A concentration-dependent increase in basal overflow was observed with prazosin 10^{-6} and 10^{-5} M (Fig 1d). At a prazosin concentration of 10^{-6} M the SEFR was estimated as the increase in fractional release over the enhanced basal efflux; an estimation at prazosin 10^{-5} M was not possible. The pattern of increase in basal and stimulation evoked overflow was identical whether prazosin was dissolved in lactic acid or HCl (see Methods).

HEAT (BE 2254) enhanced SEFR concentration-dependently (Fig 2) from 10^{-8} M, where a significant ($p < 0.001$) enhancement was observed. As in the experiments with prazosin, HEAT 10^{-6} M increased the basal efflux (see Fig 1e). At this concentration the SEFR was estimated from the elevated basal efflux. At 10^{-5} M HEAT the enhancement in basal efflux was pronounced and no estimation of SEFR was performed at this concentration.

Concentration-contraction response experiments.

Rauwolscine, prazosin, and HEAT inhibited concentration-dependently the contraction produced by NA 10^{-5} M in the CMCA (Fig 4), and the pEC_{50} -values were 7.55 ± 0.12 , 5.56 ± 0.15 and 7.11 ± 0.08 , respectively ($n=6-8$). Both prazosin and HEAT abolished the NA-induced contractions, whereas 10 ± 4 % of the response remained in presence of rauwolscine 10^{-5} M. Consistently, prazosin 3×10^{-5} M (dissolved in lactic acid) and rauwolscine 10^{-5} M both caused a contraction of the arteries amounting to about 15 % of the initial NA contraction. However, this contraction was not stable but returned to base line within a few minutes. Yohimbine, which is a stereoisomer of rauwolscine has previously been reported to contract the dog basilar

(Sakakibara et al 1982) and rabbit ear artery (Tayo 1982) by a postjunctional action. After wash out of rauwolscine, the contractile responses to NA were restored. The total inhibition of the NA contraction obtained by prazosin 3×10^{-5} M remained, however, despite frequent washing.

Comparison of pre- and postjunctional α -receptors

In order to facilitate the comparison between pre- and post-junctional effects of the drugs used, all effects of antagonists on SEFR (prejunctional effects) were expressed as percentage of the maximum enhancement in SEFR produced by HEAT, and the effects of antagonists on the contraction elicited by NA (postjunctional effects) were expressed as percentage of maximum inhibition of the NA-induced contraction obtained by HEAT (Fig 5 a and b). The effects of agonists prejunctionally were expressed as percentage of the maximum inhibitory effect obtained by oxymetazoline on the SEFR (68 %). The postjunctional effects of α -receptor agonists, measured as contractile responses in the isolated CMCA, have been determined in a previous study (Skärby et al 1983). Similarly, these values were expressed as percentage of maximum contractile effect by oxymetazoline and are given together with the corresponding prejunctional values in Fig 6.

DISCUSSION

If the maximum enhancement of SEFR caused by HEAT (487 %) is considered to reflect an almost complete blockade of prejunctional α -receptors, i.e. a state where these receptors are unstimulated by released NA, maximum activation of prejunctional receptors by agonists can be considered to reduce the ^3H -release by 90% (i.e. from 487 to 32 %, see Fig 2). Furthermore, in the present control SEFR, released NA can be regarded to cause activation of prejunctional α -receptors, which decreased the electrically evoked ^3H -release by approximately 80 % (i.e. from 487 to 100 %).

Based on these results it may be concluded that under the present experimental conditions, the inhibition of NA release in the CMCA mediated by prejunctional α -receptors is a powerful mechanism by which the transmitter efflux can be regulated.

When the agonist clonidine is more potent than phenylephrine, and the antagonist rauwolscine is more potent than prazosin, the receptor has been suggested to be of α_2 -type. When the reverse was true, the receptor has been proposed to be of α_1 -type (Langer & Shepperson 1981, Starke 1981, Timmermans & van Zwieten 1981, McGrath 1982).

In a previous study (Skärby et al 1983) the order of potency of agonists to produce contractile responses in the CMCA was: clonidine $\geq$ oxymetazoline $>$ NA $>$ phenylephrine. These findings indicate that the postjunctional α -receptors in this vessel are predominantly of α_2 -type. This is further supported by the present study in which rauwolscine was much more potent than prazosin to inhibit the contraction elicited by NA.

In the present study, the relative order of potency of agonists to inhibit SEFR was: clonidine $\approx$ oxymetazoline $>$ phenylephrine, and the antagonist rauwolscine was much more potent than prazosin. This is in good agreement with results obtained prejunctionally in a number of different tissues (see e.g. Sullivan & Drew 1980, Docherty & Starke 1982, Frankhuyzen & Mulder 1982, Reichenbacher et al 1982) and suggests that the prejunctional α -receptors are mainly of α_2 -type. In contrast to these studies and the

investigations by Davey (1980) and Raiteri et al (1983), 10^{-7} M prazosin increased (41 ± 6 %, $n=8$) SEFR significantly in the present study.

Based on results obtained with radioligand binding technique Hoffman & Lefkowitz (1980) and Andersson et al (1984), suggested that prazosin in the concentration 10^{-7} M occupies almost exclusively α_1 -receptors with virtually no effect on α_2 -receptors.

Prejunctional α_1 -receptors has been suggested to be present in the rat heart (Kobinger & Pichler 1980, 1982, Docherty 1983). In order to investigate if part of the inhibition of ^3H -release in the CMCA was mediated by a population of α_1 -receptors, the effects of prazosin on the concentration-inhibition (CI) curve for clonidine and phenylephrine were examined and compared to those of rauwolscine (Fig 3). Rauwolscine (10^{-9} M) caused a significant shift of the CI-curve for clonidine towards higher concentrations, indicating that these drugs interfered with the same type of receptor and that this receptor is of α_2 -type. Prazosin (10^{-7} M) significantly attenuated the effect of 10^{-8} M clonidine and appeared also to shift the CI curve for clonidine, suggesting that prazosin may interact with the same receptor population. The lack of significant effects of prazosin and rauwolscine on the CI-curve for phenylephrine, implies that none of these antagonists interfered with the same site as phenylephrine. In the rat heart the prejunctional α -receptors were stimulated by an α_1 -receptor agonist (ST 587), and in variance with the present study this effect was more potently blocked by prazosin than by rauwolscine (Kobinger and Pichler 1982). Furthermore, Docherty (1983) found in the same tissue that prazosin was more effective in antagonizing the prejunctional effects caused by cirazoline (α_1 -receptor agonist) than in blocking the prejunctional effects of xylazine (α_2 -receptor agonist), whereas the reverse was true for yohimbine (α_2 -receptor antagonist). The effects of prazosin and phenylephrine on SEFR in the present study therefore do not seem to favor the existence of a population of prejunctional α_1 -receptors, but rather suggest that the prejunctional α_2 -receptors in the CMCA can be blocked by unusually low concentrations of prazosin. However, as the shift of the clonidine CI-curve by

prazosin was not statistically significant (only the effect of clonidine 10^{-8} M was significantly affected) it can not be excluded that the increase in SEFR by prazosin 10^{-7} M was nonspecific and unrelated to blockade of prejunctional α -receptors.

HEAT (BE 2254) has previously been found to be a potent antagonist on both prejunctional (α_2) and postjunctional (α_1) α -receptors in the rabbit pulmonary artery (Göthert et al 1981). Hicks (1981) suggested that HEAT was 185 times more potent at pre- than at postjunctional α_2 -receptors in the cardiovascular system of the rat. He therefore proposed that these types of α_2 -receptors were pharmacologically different. In contrast to the study by Hicks (1981) the pre- and postjunctional effects of HEAT in the present study were almost identical (see Fig 5a). This indicates that HEAT lacks the ability to discriminate between the pre- and postjunctional α -receptors in the CMCA.

The fact that both prazosin and phenylephrine did not exhibit higher potency on post- than prejunctional receptors (but rather the contrary) further emphasizes the advantage of classifying α -receptors pharmacologically rather than depending on the anatomical localization.

With the exception of clonidine, which appeared to have a higher intrinsic activity pre- than postjunctionally, all the drugs studied had similar concentration-effect curves on pre- and postjunctional sites (see Figs 5 a,b and 6). Furthermore the ratios of EC_{50} -values pre- and postjunctionally of rauwolscine, oxymetazoline, and clonidine (1.12, 0.81, and 0.93, respectively) were all close to unity, suggesting that these drugs have similar potency on the two sites.

These results suggest that pre- and postjunctional α_2 -receptors are not separable with the presently used pharmacological tools. This conclusion is supported by recent results obtained in pithed rats in vivo by Paciorek & Shepperson (1983), showing that pre- and postjunctional α_2 -receptors did not differ in their sensitivity to α -receptor-antagonists.

REFERENCES

- ANDERSSON, K.-E., LARSSON, B. & SJÖGREN, C. 1984. Characterization of α -adrenoceptors in the female rabbit urethra. *Br J Pharmacol* 81:293-300.
- DAVEY, M.J. 1980. Relevant features of the pharmacology of prazosin. *J Cardiovasc Pharmacol* (suppl 3) 2:s287-s301.
- DE MEY, J. & VANHOUTTE, P.M. 1981. Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 48:875-884.
- DOCHERTY, J.R. 1983. An investigation of presynaptic α -adrenoceptor subtypes in the pithed rat heart. *Br J Pharmacol* 78:655-657.
- DOCHERTY, J.R. & STARKE, K. 1982. An examination of the pre- and postsynaptic α -adrenoceptors involved in neuroeffector transmission in rabbit aorta and portal vein. *Br J Pharmacol* 76:327-335.
- ELLIOTT, H.L. & REID, J.L. 1983. Evidence for postjunctional vascular α_2 -adrenoceptors in peripheral vascular regulation in man. *Clin Sci* 65:237-241.
- FRANKHUYZEN, A.L. & MULDER, A.H. 1982. Pharmacological characterization of presynaptic α -adrenoceptors modulating ^3H noradrenaline and ^3H 5-hydroxytryptamine release from slices of the hippocampus of the rat. *Eur J Pharmacol* 81:97-106.
- GLUSA, E. & MARKWARDT, F. 1983. Characterization of postjunctional α -adrenoceptors in isolated human femoral veins and arteries. *Naunyn-Schmiedeberg's Arch Pharmacol* 323:101-105.
- GÖTHERT, M., NOLTE, J. & WEINHEIMER, G. 1981. Preferential blockade of postsynaptic α -adrenoceptors by BE 2254. *Eur J Pharmacol* 70:35-42.
- HICKS, P.E. 1981. Antagonism of pre and postsynaptic α -adrenoceptors by BE 2254 (HEAT) and prazosin. *J Auton Pharmacol* 1:391-397.

- HOFFMAN, B.B. & LEFKOWITZ, R.J. 1980. An assay for alpha-adrenergic receptor subtypes using ^3H dihydroergocryptine. *Biochem Pharmacol* 29:452-454.
- HORN, P.T., KOHLI, J.D., LISTINSKY, J.J. & GOLDBERG., L.I. 1982. Regional variation in alpha adrenergic receptors in the canine resistance vessels. *Naunyn Schmiedeberg's Arch Pharmacol* 318:116-172.
- HÖGESTÄTT, E.D. & ANDERSSON, K.-E. 1984. On the postjunctional α -adrenoceptors in rat cerebral and mesenteric arteries. Submitted for publication.
- HÖGESTÄTT, E., ANDERSSON, K.-E. & EDVINSSON, L. 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol Scand* 117:49-61.
- KOBINGER, W. & PICHLER, L. 1980. Investigation into different types of post- and presynaptic α -adrenoceptors at cardiovascular sites in rats. *Eur J Pharmacol* 65:393-402.
- KOBINGER, W. & PICHLER, L. 1982. Presynaptic activity of the imidazoline derivative ST 587, a highly selective α_1 -adrenoceptor agonist. *Eur J Pharmacol* 203-206.
- LANGER, S.Z. 1981. Presynaptic regulation of the release of catecholamines. *Pharmacol. rev.* 32:337-362.
- LANGER, S.Z. & MEDGETT, I.C. 1982. Stimulation of α_2 -adrenoceptors but not of sympathetic nerves constricts cat isolated perfused middle cerebral arteries. *Br J Pharmacol* 77:478. (Proc. suppl.).
- LANGER, S.Z. & SHEPPERSON, N.B. 1981. Subclassification of α -adrenoceptor into α_1 - and α_2 -categories: relevance to antihypertensive therapy. New trends in arterial hypertension. In *Interm Symposium No 17* (ed M. Worcel et al) Elsevier/North Holland Biochemical Press. pp 73-85

- MCGRATH, J.C. 1982. Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem Pharmacol* 31:467-484.
- MEDGETT, I.C. & LANGER, S.Z. 1983. Characterization of smooth muscle α -adrenoceptors and of responses to electrical stimulation in the cat isolated perfused middle cerebral artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 323:24-32.
- PACIOREK, P.M. & SHEPPERSON, N.B. 1983. A study of pre- and postsynaptic α_2 -adrenoceptors in the pithed rat. *Br J Pharmacol* (suppl.) 79:225p.
- RAITERI, M., MURA, G. & VERSACE, P. 1983. Functional evidence for two stereochemically different α_2 -adrenoceptors regulating central norepinephrine and serotonin release. *J Pharmacol Exp Ther* No 3 224:679-684.
- REICHENBACHER, D., REIMANN, W. & STARKE, K. 1982. α -Adrenoceptor-mediated inhibition of noradrenaline release in rabbit brain cortex slices. Receptor properties and role of the biophasic concentration of noradrenaline. *Naunyn-Schmiedeberg's Arch Pharmacol* 319:71-77.
- RUFFOLO, R.R., WADDEL, J.E. & YADEN, E.L. 1981. Postsynaptic alpha-adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of α_2 -adrenergic receptors in rat aorta. *J Pharmacol Exp Ther.* 217:235-240.
- RUFFOLO, R.R., WADDEL, J.E. & YADEN, E.J. 1982. Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *J Pharmacol Exp Ther* 221:309-314.
- SAKAKIBARA, Y., FUJIWARA, M. & MURAMATSU, I. 1982. Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 319:1-7.

- SKÄRBY, T., ANDERSSON, K.-E. & EDVINSSON, L. 1981. Characterization of the postsynaptic α -adrenoceptor in isolated feline cerebral arteries. *Acta Physiol Scand* 112:105-107.
- SKÄRBY, T.V.C., ANDERSSON, K.-E. & EDVINSSON, L. 1983. Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand* 117:63-73.
- STARKE, K. 1981. α -adrenoceptor subclassification. *Rev. Physiol Biochem Pharmacol* 88: 199-236.
- STEEN, S., SKÄRBY, T.V.C., NORGREN, L. & K.-E. ANDERSSON. 1984a. Pharmacological characterization of postjunctional α -adrenoceptors in isolated human omental arteries and veins. *Acta Physiol Scand*. 120:109-116
- STEEN, S., SJÖBERG, T., SKÄRBY, T., NORGREN, L. & ANDERSSON, K.-E. 1984b. Pharmacological characterization of postjunctional α -adrenoceptors in isolated human groin arteries and veins. *Acta physiol Scand*. Accepted for publication.
- STEVENS, M.J. & MOULDS, R.F.W. 1982. Are the pre- and postsynaptic α -adrenoceptors in human vascular smooth muscle atypical. *J. Cardiovasc. Pharmacol* 4:s129-133.
- SULLIVAN, A.T. & DREW, G.M. 1980. Pharmacological characterization of pre- and postsynaptic α -adrenoceptors in dog saphenous vein. *Naunyn-Schmiedeberg's Arch Pharmacol* 314:249-258.
- TAYO, F. M. 1982. Agonist action of yohimbine on the perfused rabbit central ear artery. *Blood Vessels* 19:197-202.
- TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. 1981. The postsynaptic α_2 -adrenoceptor. *J Auton Pharmacol* 1:171-183.
- TODA, N. 1983. Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. *J Pharmacol Exp Ther* No 3 226:861-868.

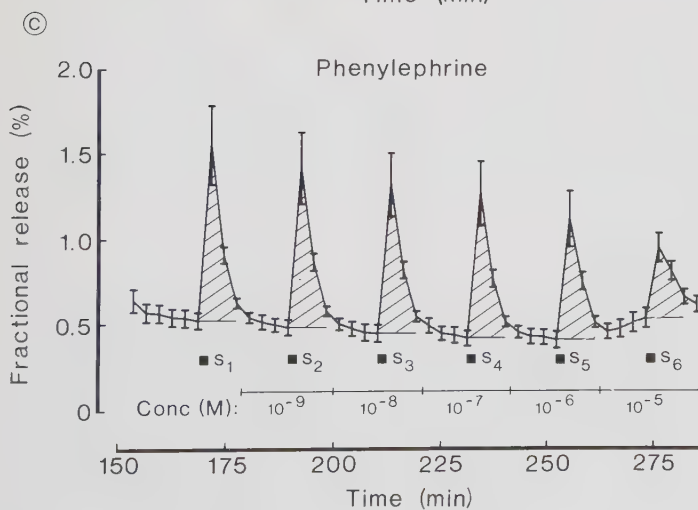
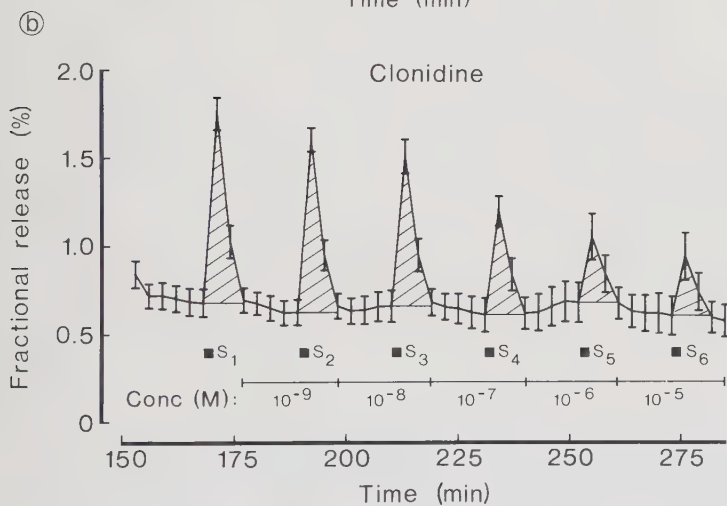
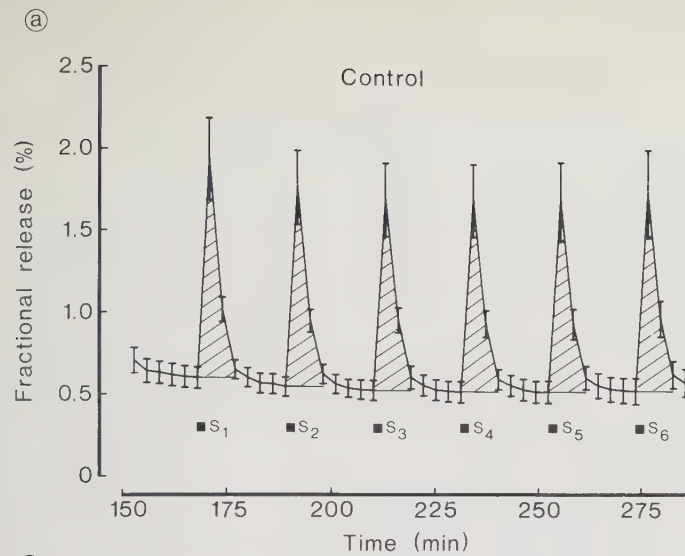
VAN BRUMMELEN, P., VERMEY, P., TIMMERMANS, P.B.M.W.M. & VAN
ZWIETEN, P.A. 1982. Preliminary evidence for a postsynaptic α_2 -
adrenoceptor in the vasculature of the human forearm. Proc. B.P.S.
8th-10th Sept 1982.134-135p.

Table 1. Effects of increasing number of stimulation periods on the ratio S_n/S_1 .

D r u g	n	S t i m u l a t i o n				
		S_2/S_1	S_3/S_1	S_4/S_1	S_5/S_1	S_6/S_1
No drug	13	0.95 ± 0.02	0.96 ± 0.03	0.95 ± 0.03	0.95 ± 0.04	1.00 ± 0.05
^x Prazosin 10^{-7} M	6	0.98 ± 0.05	0.92 ± 0.03	0.91 ± 0.04	0.90 ± 0.07	0.87 ± 0.05
^x Rauwolscine 10^{-9} M	5	0.97 ± 0.03	0.97 ± 0.03	0.96 ± 0.05	0.96 ± 0.05	0.92 ± 0.05

S_n = stimulation evoked fractional release in the 2nd, 3rd, 4th, 5th or 6th stimulation period.

^xThe drug was in this concentration present during all six stimulation periods ($S_1 - S_6$).



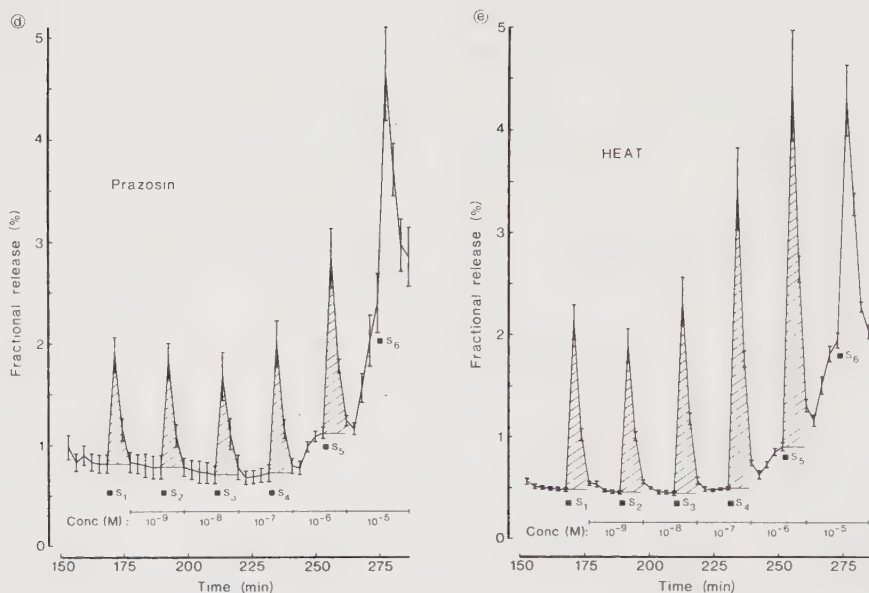
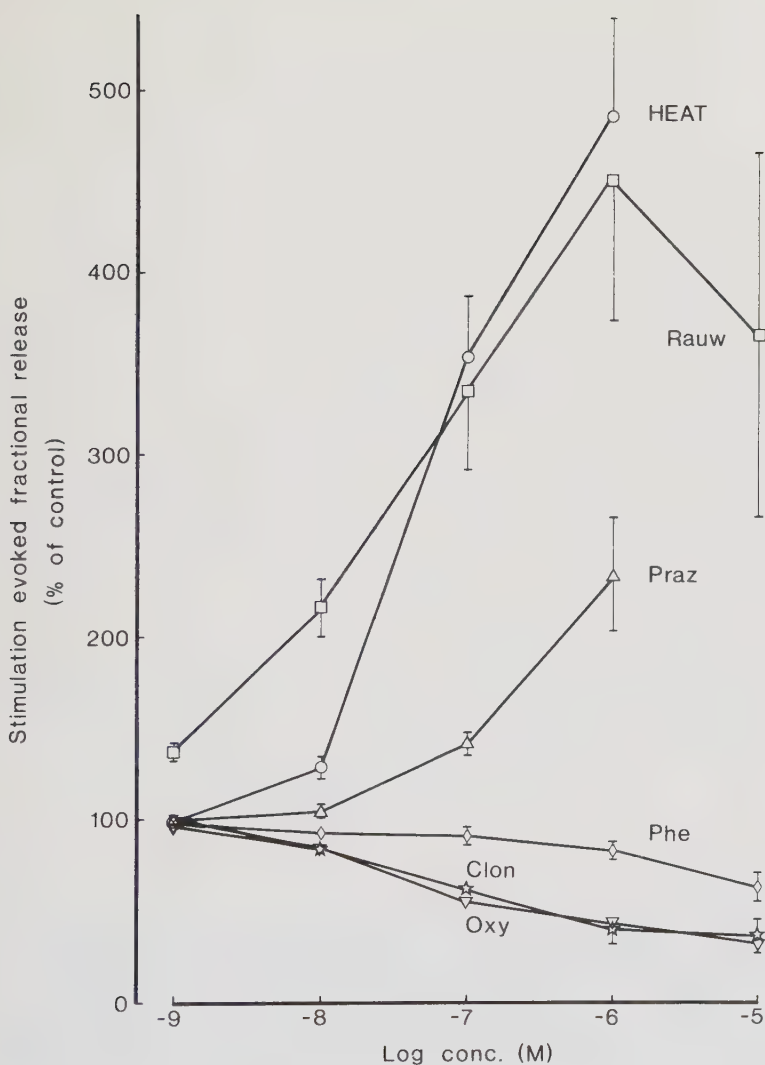


Fig 1 ^3H -overflow from feline middle cerebral arteries stimulated electrically in the absence and presence of drugs. The abscissa shows time after onset of perfusion. The tissue was stimulated 6 times at 2 Hz for 2.5 minutes at points indicated (S_1 - S_6). Each point represents mean $\pm$ SE of 4-13 determinations. Hatched areas indicate the estimated stimulation evoked fractional release.

- 6 consecutive control stimulations in absence of test drugs.
- Effects of clonidine.
- Effects of phenylephrine.
- Effects of prazosin.
- Effects of HEAT (BE 2254).



g 2 The effects of rauwolscine (Rauw □) HEAT (○), prazosin (Praz △) phenylephrine (Phe ◇), clonidine (Clon ☆), and oxymetazoline (Oxy ▽) on stimulation evoked fractional release in the feline middle cerebral artery. Each point represents mean $\pm$ SE of 4-7 determinations.

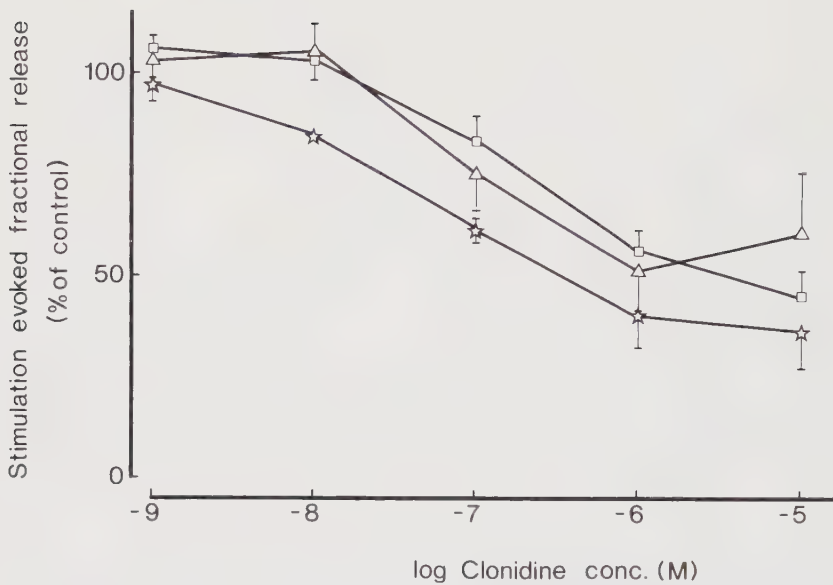


Fig 3 Concentration-effect curves to clonidine (☆) in the presence of rauwolscine 10^{-9} M (□) and prazosin 10^{-7} M (△). Where used, rauwolscine or prazosin were present at least 18 min before and during the 1st stimulation period (S_1) and continuously present during the rest of the experiment. Each value represents mean $\pm$ SE of 3 - 5 determinations.

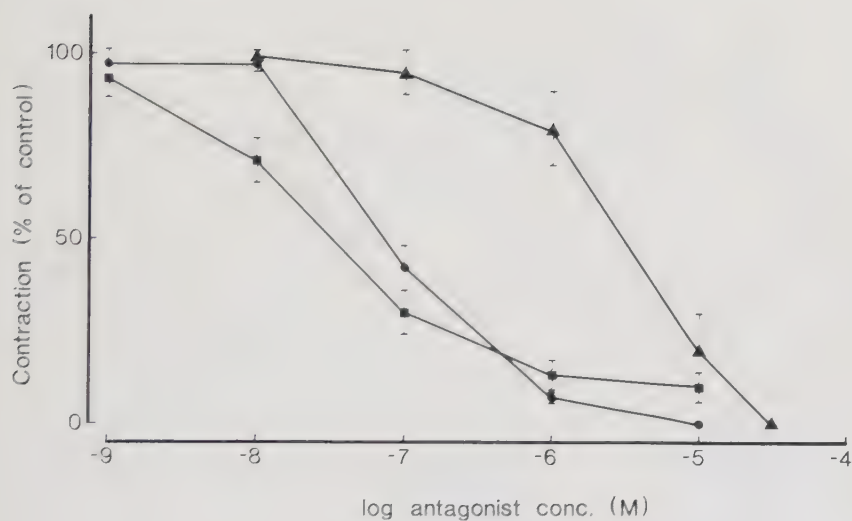


Fig 4 Effects of rauwolscine (■) HEAT (BE 2254) (●) and prazosin (▲) on the contractile responses to noradrenaline (NA) (10^{-5} M). The effects are expressed as percentage of the control contraction before addition of antagonists. The drugs were present at least 15 min prior to the addition of NA 10^{-5} M and the buffer contained propranolol 3×10^{-7} M. Each value represents mean $\pm$ SE of 6 - 8 determinations.

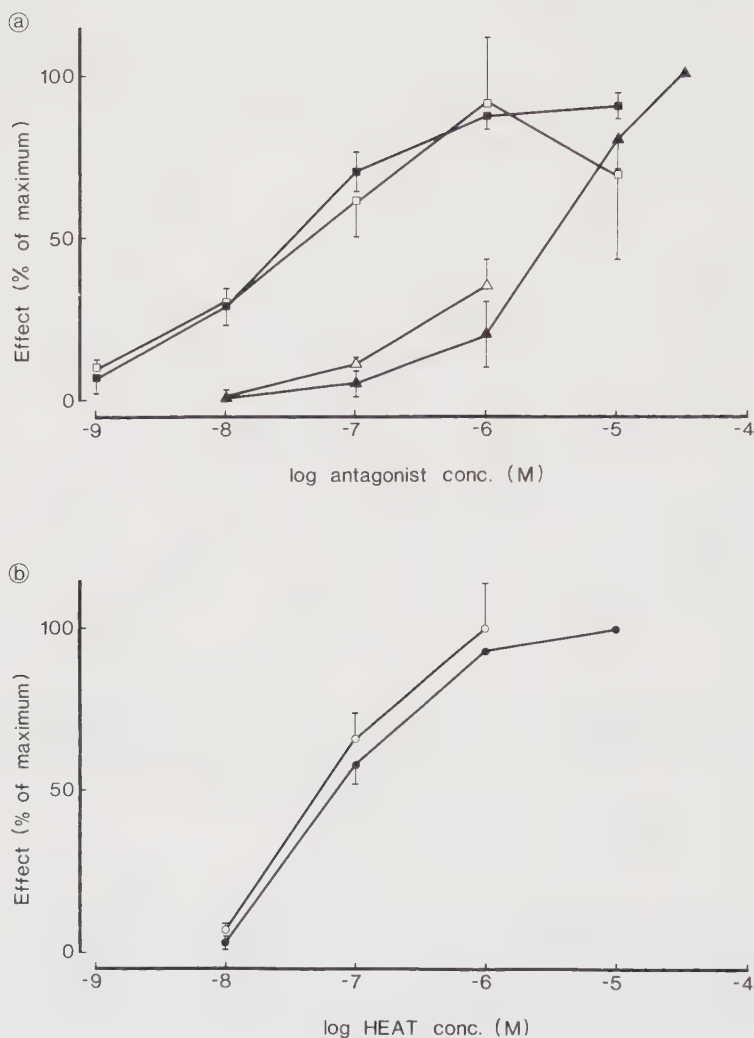


Fig 5 Effects of a) rauwolscine ($\blacksquare$) and prazosin ($\blacktriangle$) and b) HEAT ($\bullet$) on the contractile responses to noradrenaline 10^{-5} M (filled symbols), and the effects of the same antagonists on stimulation evoked fractional release (SEFR) (open symbols). Both effects are expressed as percentage of the maximum response caused by HEAT with respective experimental procedure (see "Results"). Each value represents mean $\pm$ SE of 5 - 8 determinations.

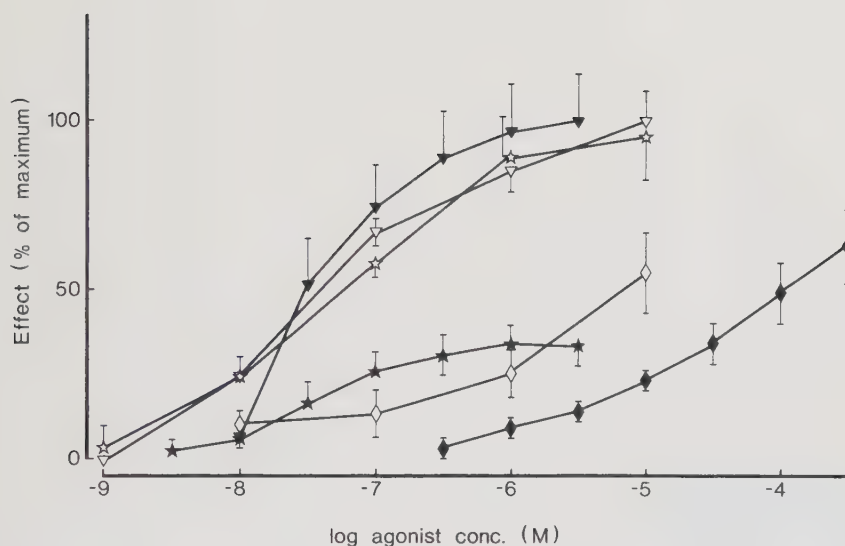


Fig 6 Contractile responses (filled symbols) by clonidine (★), oxymetazoline (▼) and phenylephrine (◆) and the effects of the same agonists on stimulation evoked fractional release (open symbols). In the contraction experiments the drugs were added cumulatively and the buffer contained 10^{-6} cocaine and 10^{-7} M propranolol. All effects are expressed as percentage of maximum response caused by oxymetazoline in respective test system. Each value represents mean $\pm$ SE of 4 - 8 determinations.

IV

Influence of extracellular calcium and calcium antagonists on contractions induced by potassium and prostaglandin $F_{2\alpha}$ in isolated cerebral and mesenteric arteries of the cat

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- 1 The effects of a number of calcium antagonists (diltiazem, nifedipine, nimodipine and verapamil) have been studied on feline isolated pial arteries contracted by potassium (127 mM) or prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$, 2.5 μM) and mesenteric arteries contracted by potassium (127 mM).
- 2 Withdrawal of Ca^{2+} from the extracellular medium for 30 min reduced the contractile response to potassium in cerebral vessels by 92% and in mesenteric vessels by 96%. Subsequent addition of Ca^{2+} caused reproducible contractions which were inhibited by both nifedipine and nimodipine.
- 3 The four calcium antagonists relaxed the isolated middle cerebral artery contracted either by potassium or $\text{PGF}_{2\alpha}$, and mesenteric arteries contracted by potassium, in the following order of potency: nimodipine > nifedipine > verapamil > diltiazem.
- 4 Nimodipine was more potent than nifedipine in cerebral arteries, and more potent in cerebral than in mesenteric arteries. Otherwise, the potassium-contracted cerebral and mesenteric vessels showed no major differences in sensitivity to calcium antagonists.

Introduction

Cerebrovascular smooth muscle tone is regulated by a complex system of input mechanisms by vasomotor nerves, hormones and local physical and chemical factors, all of which have been thoroughly described for the peripheral circulation (Johansson, 1978). These factors implicate a final common step in the process linking membrane excitation and contraction; namely, the concentration of calcium ions in the vicinity of the contractile proteins. This calcium ion concentration determines the degree of activation of the vascular smooth muscle cells. Many factors may trigger the release of calcium ions from intracellular stores (such as mitochondria, sarcoplasmic reticulum) and/or increase the calcium ion influx from the extracellular medium through specific membrane channels (Bolton, 1979).

Several approaches have been used to examine these fundamental processes and to estimate the relative importance of different calcium pools for the mechanism of activation. Among the tools used are different antagonists of the calcium involved in the excitation-contraction coupling (Fleckenstein, 1977). Although some reports have demonstrated the necessity of extracellular calcium for cerebrovas-

cular muscle contraction (Betz & Csornai, 1978; Toda, 1976), knowledge of the role of extracellular calcium for activation and relaxation in the vascular bed is still limited.

In the present study, we have examined the influence of variations in extracellular calcium concentration on the contractions induced by either potassium or prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) in feline pial or mesenteric vessels. Furthermore, the action of four different calcium antagonists was examined and compared.

Methods

Preparation and mounting

Cats of either sex were anaesthetized with sodium pentobarbitone (30 mg/kg, i.p.) and killed by exsanguination and decapitation. The skull was opened, the brain removed, placed in a Petri dish and soaked in a cold Krebs buffer solution. The middle cerebral arteries were subsequently removed and placed in a glass beaker containing the same buffer. Immediately after, a part of the mesentery, containing arteries of

similar diameter to the cerebral vessels, was removed. With the aid of an operating microscope, arterial segments were dissected free and placed in a glass beaker containing the cold buffer solution. Part of the material was used immediately in the experiments, while the rest was stored in a refrigerator (+4°C) for up to 24 h. There was no difference in response to potassium or PGF_{2α} between fresh and day-old arteries.

Vessel segments (300–400 µm in diameter, 2–4 mm long) were mounted in 5 ml temperature-controlled tissue baths (one segment in each bath) containing a system of L-shaped metal holders for recording isometric circular contractions (Högestätt, Andersson & Edvinsson, 1983). Contractions were measured by means of Grass FT-03 transducers, amplified and recorded on a Grass polygraph.

The baths contained a Krebs buffer solution of the following composition (mM): NaCl 119, KCl 4.6, CaCl₂ 1.5, MgCl₂ 1.2, NaHCO₃ 15, NaH₂PO₄ 1.2 and glucose 11.0. A solution with a high potassium content (127 mM) was obtained by replacing NaCl with equivalent amounts of KCl. In experiments involving a calcium-free solution, CaCl₂ was omitted from the normal Krebs solution and EGTA 10 µM added. The bath and stock solutions were kept at 37°C and aerated continuously with a mixture of 95% O₂ and 5% CO₂ to maintain a pH of 7.4. Chemicals used were of analytical grade.

Experimental procedure

Shortly after the arterial preparations had been mounted in the organ baths, the vessels were subjected to a load of 5 mN. Test drugs were administered when a stable base line was reached, usually after 60 to 90 min. Contractions were induced by potassium (127 mM), resulting in a response which was stable for more than 30 min.

In some experiments the vessels were contracted by the addition of PGF_{2α} (2.5 µM) which produced a response that was stable for at least 30 min. The calcium antagonists were added to the organ baths in a cumulative manner in concentrations ranging from 0.1 nM to 100 µM. Subsequently, log concentration-response curves were constructed from the data obtained. Calculations were made of the IC₅₀ value (the concentration of drug producing half maximum inhibition) and the I_{max} value (the maximum inhibitory response produced, expressed as a percentage of the stable contraction induced by either PGF_{2α} or a high concentration of potassium). Vessels which did not respond to potassium or PGF_{2α} with reproducible contractions were rejected.

Drugs

The following drugs were used: diltiazem (Tanabe);

nifedipine (Bayer); nimodipine (Bayer); verapamil (Knoll); and PGF_{2α} (Amoglandin, Astra). These substances were dissolved in 0.9% w/v NaCl solution (saline). All concentrations are expressed as the final molar concentrations in the bath. Nifedipine and nimodipine were kept in a dark environment due to the possibility of light-induced decomposition.

Results

Influence of extracellular calcium

The contractile responses to potassium of both pial and mesenteric vessels were concentration-dependent and reproducible in the standard Krebs solution. The threshold concentration for contraction was approximately 15–20 mM in cerebral vessels and 5–10 mM higher in mesenteric arteries; contraction was almost maximal at 60 mM for both types of vessel. Exposure of cerebral and mesenteric arteries to a calcium-free Krebs solution for 30 min reduced the contractile effect of potassium (127 mM) to 8 ± 2% and 4 ± 1% of the controls respectively. The vessel segments contracted promptly upon re-administration of calcium; this response again being concentration-related with the maximum effect at 4 mM calcium (Figure 1a and 1b).

In the absence of extracellular calcium, nifedipine (0.3 µM) and nimodipine (0.21 µM) respectively caused a further decrease in the response induced by potassium to 3 ± 1% and 2 ± 1% in cerebral arteries ($P < 0.001$; Student's *t* test), and that of mesenteric arteries to 0%. These two calcium antagonists markedly inhibited the contraction produced by re-introducing calcium into the extracellular medium (Figures 1a and b).

Effects of calcium antagonists

Cerebral arteries: The four calcium antagonists relaxed pial arteries, contracted by either 2.5 µM PGF_{2α} or 127 mM potassium, in the following order of potency: nimodipine > nifedipine > verapamil > diltiazem (Table 1). Vessels contracted either by PGF_{2α} or potassium were almost completely relaxed (Figures 2a and b). There was a slight, but statistically significant difference ($P < 0.05$) in potency between nifedipine and nimodipine in relaxing potassium-contracted arteries, the latter being approximately four times more potent (Table 1). However, the IC₅₀ value for relaxation of PGF_{2α}-contracted vessels by nimodipine was about 10 times lower ($P < 0.001$) than that of nifedipine (Table 1). The IC₅₀ values for the calcium antagonists to relax the arteries were otherwise similar whether the vessels had been contracted by potassium or by PGF_{2α} (Table 1).

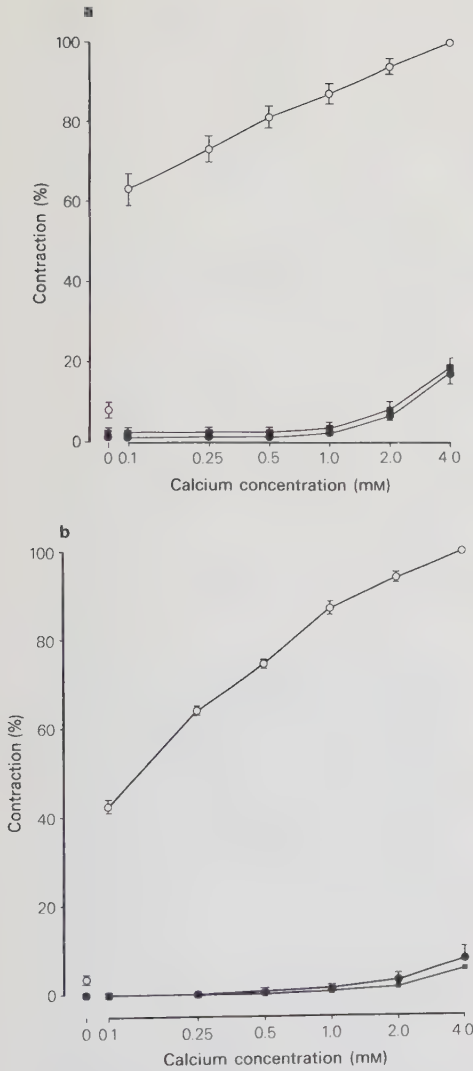


Figure 1 Responses of feline isolated (a) middle cerebral and (b) mesenteric arteries to increasing concentrations of calcium after exposure to a Krebs solution containing zero calcium. Data are given as percentage of maximum. Potassium (127 mM) was added in the absence of drug (○), and in the presence of nifedipine (0.3 μM, ●), or nimodipine (0.21 μM, ■). Each point represents the mean value of 6 determinations; vertical lines show s.e.mean.

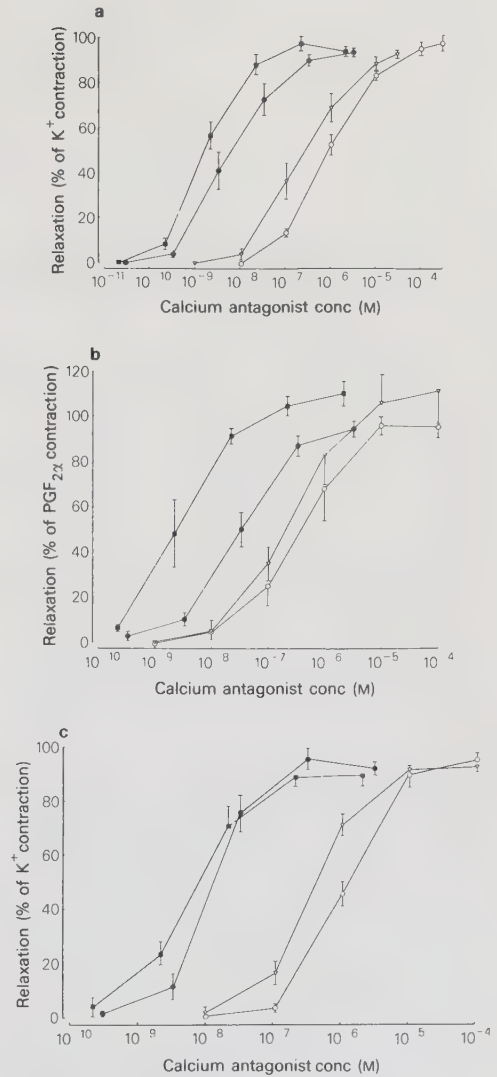


Figure 2 Inhibitory effect of nimodipine (■), nifedipine (●), verapamil (Δ) and diltiazem (○) each added cumulatively, on the contractile responses to (a) potassium (127 mM) or (b) prostaglandin F_{2α} (2.5 μM) on feline isolated middle cerebral arteries and (c) on feline mesenteric arteries contracted by 127 mM potassium. Each point represents the mean value of 5 to 12 determinations; vertical lines show s.e.mean. For further details see Tables 1 and 2.

Table 1 Relaxation of isolated middle cerebral arteries of the cat by four different calcium antagonists

Agent	n	Potassium-contracted vessels		n	PGF _{2α} -contracted vessels	
		IC ₅₀ (M)	I _{max} (%)		IC ₅₀ (M)	I _{max} (%)
Nimodipine	8	$1.9 \pm 1.5 \times 10^{-9}$	97 ± 3	7	$2.9 \pm 1.3 \times 10^{-9}$	104 ± 4
Nifedipine	8	$7.2 \pm 2.3 \times 10^{-9}$	93 ± 2	9	$2.8 \pm 1.2 \times 10^{-8}$	95 ± 3
Verapamil	5	$2.0 \pm 1.7 \times 10^{-7}$	93 ± 2	6	$3.3 \pm 1.5 \times 10^{-7}$	111 ± 18
Diltiazem	6	$1.1 \pm 1.4 \times 10^{-6}$	98 ± 4	8	$3.7 \pm 2.0 \times 10^{-7}$	96 ± 4

Contraction was induced either by 127 mM potassium buffer (9.0 ± 0.8 mN, $n = 26$) or by $2.5 \mu\text{M}$ prostaglandin F_{2α} (PGF_{2α}, 7.2 ± 2.3 mN, $n = 30$). Mean values ± s.e. mean are given (n = number of arteries examined). I_{max} is the maximum inhibitory response of the vessel, expressed as a percentage of the induced contraction.

Table 2 Relaxation of mesenteric arteries of the cat contracted by a high potassium (127 mM) buffer solution

Agent	n	IC ₅₀ (M)	I _{max} (%)
Nimodipine	7	$1.0 \pm 2.6 \times 10^{-8}$	90 ± 4
Nifedipine	6	$1.6 \pm 0.2 \times 10^{-8}$	96 ± 4
Verapamil	5	$5.4 \pm 0.8 \times 10^{-7}$	92 ± 2
Diltiazem	5	$2.8 \pm 1.5 \times 10^{-6}$	95 ± 3

The contractions were 20.3 ± 5.3 mN ($n = 23$). I_{max} is the maximum inhibitory response of the vessel, expressed as a percentage of the induced contraction. Mean values ± s.e. mean are given (n = number of arteries examined).

Mesenteric arteries: In mesenteric arteries, potassium induced strong persistent contractions (20.3 ± 1.9 mN) of the vessels. The calcium antagonists relaxed potassium-contracted vessels concentration-dependently with the same order of potency as found in cerebral vessels, *i.e.* nimodipine > nifedipine > verapamil > diltiazem (Figure 2c).

The degree of relaxation and the mean IC₅₀ values of the calcium antagonists are given in Table 2. The IC₅₀ and I_{max} values are similar to those obtained in pial vessels, with the exception of nimodipine which appeared to have a lower IC₅₀ value (Student's *t* test, $P < 0.001$) in the latter. Contractions induced by PGF_{2α} in mesenteric arteries were not stable enough to allow further analysis.

Discussion

Contractions induced in vascular smooth muscle by a high concentration of potassium are generally believed to be highly dependent on the concentration of extracellular calcium; these contractions are markedly decreased by the removal of calcium from the extracellular milieu (Briggs, 1962; Hinke, 1965; Hudgins & Weiss, 1968; van Breemen, 1969). In the

present investigation, the concentration threshold for potassium to produce contraction was about 15 mM. This threshold seems to be lower in human cerebral arteries, where it was found recently to be about 10 mM (Brandt, Andersson, Edvinsson & Ljunggren, 1981). Potassium concentrations as high as 127 mM cause total depolarization of the cell membrane and induce very reproducible contractions.

The contractile response of vascular smooth muscle to potassium consists of two phases: one initial and rapid; the other slow in onset and long-lasting. Both phases are considered to depend on calcium associated with extracellular or surface membrane pools (van Breemen, Farinas, Gerba & McNaughton, 1972; Deth & van Breemen, 1974). Calcium inflow is believed to occur through potential-sensitive channels in the membrane; these channels can be blocked by calcium antagonists (Bolton, 1979).

The present results showed that the responses to potassium in both cerebral and mesenteric vessels were decreased to less than 10% after 30 min in a calcium-free medium (Figure 1). These results illustrate the dependency on extracellular calcium for contraction, emphasised further by the fact that replacement of calcium induces contractions which reach their initial tension value at a concentration of 4 mM. Both nifedipine and nimodipine, as expected,

suppressed the contractile effect caused by re-introducing calcium into the bathing fluid. The small contractions induced by potassium in the absence of calcium were further reduced by nifedipine and nimodipine, suggesting that these drugs not only inhibit the influx of extracellular calcium during the depolarization by potassium, but may also have intracellular effects (Church & Zsotér, 1980). They may attenuate the release of intracellularly bound calcium or interact with, for example, calmodulin (Boström, Ljung, Mårdh, Forsén & Thulin, 1981). Data supporting an additional site of action of calcium antagonists in vascular smooth muscle have been obtained in studies on human mesenteric (Mikkelsen, Andersson & Lederballe Pedersen, 1979) and pial vessels (Brandt *et al.*, 1981). Another calcium antagonist, verapamil, has been shown recently to have its main site of action at the inner side of the cell membrane of rat heart cells, a site different from that of Mn^{2+} (Payet, Schanne, Ruiz-Ceretti & Demers, 1980). Thus, calcium antagonists may well turn out to have several sites of action.

A number of previous studies have demonstrated the contractile effect of $PGF_{2\alpha}$ both in cerebral (Toda, 1976; Edvinsson, Hardebo, McCulloch & Owman, 1978; Uski, Edvinsson & Owman, 1981) and peripheral vessels (Nakano, 1968; Greenberg & Sparks, 1969). The maximum contractile effect of $PGF_{2\alpha}$ corresponds well with that of potassium in pial arteries although a longer time is taken to reach the maximum response. In the present, as well as in previous studies (Edvinsson *et al.*, 1978), the contraction induced by $PGF_{2\alpha}$ on the feline middle cerebral artery remained stable for at least 30 min and this allowed relaxation to be studied. The four calcium antagonists relaxed both potassium- and $PGF_{2\alpha}$ -contracted vessels concentration-dependently and completely (Figure 2). This is in contrast to recent findings on human cerebral arteries (Brandt *et al.*, 1981), where potassium-contracted vessels relaxed almost completely, while $PGF_{2\alpha}$ -

contracted arteries were relaxed by only 60%. Shimizu, Ohta & Toda (1980) have also shown that in dog cerebral arteries, verapamil relaxed potassium-contracted arteries more than $PGF_{2\alpha}$ -contracted vessels. This difference may indicate that, in human and dog cerebral vessels but not in feline vessels, the $PGF_{2\alpha}$ -induced contractions are less dependent on the influx of extracellular calcium across the arterial smooth muscle cell membrane, than contractions evoked by depolarization of the vessels by potassium.

The potency differences between the calcium antagonists accord with the literature (Fleckenstein, 1977; Mikkelsen *et al.*, 1979; Shimizu *et al.*, 1980). Nimodipine had the highest potency, and diltiazem the lowest; there were no differences between the maximum vasodilator effects obtained. Nimodipine and nifedipine have been suggested as selective calcium antagonists for cerebral vessels (Towart & Kazda, 1980) and have been shown to have greater effects on cerebral than peripheral arteries in the dog (Allen & Banghart, 1979; Shimizu *et al.*, 1980). A significant difference in potency on pial vessels between nimodipine and nifedipine was observed in the present investigation, irrespective of the contractile agent used (potassium or $PGF_{2\alpha}$); nimodipine being 4–10 times more potent than nifedipine. On the other hand, nifedipine had a similar potency in cerebral and mesenteric arteries contracted by potassium and in mesenteric arteries, nifedipine and nimodipine were equipotent. Thus, nimodipine had a certain degree of selectivity for cerebral vessels giving further support to the view that species and regional differences exist in the action and potency of calcium antagonists.

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References

- ALLEN, G.S. & BANGHART, S.B. (1979). Cerebral arterial spasm: Part 9. In vitro effects of nifedipine on serotonin-, phenylephrine-, and potassium-induced contractions of canine basilar and femoral arteries. *Neurosurgery*, **4**, 37–42.
- BETZ, E. & CSORNAI, M. (1978). Action and interaction of perivascular H^+ , K^+ and Ca^{++} on pial arteries. *Pflügers Arch.*, **374**, 67–72.
- BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.
- BOSTRÖMS, S.-L., LJUNG, B., MÅRDH, S., FORSÉN, S. & THULIN, E. (1981). Interaction of the antihypertensive drug felodipine with calmodulin. *Nature*, **292**, 777–778.
- BRANDT, L., ANDERSSON, K.-E., EDVINSSON, L. & LJUNGGREN, B. (1981). Effects of extracellular calcium and of calcium antagonists on the contractile responses of isolated human pial and mesenteric arteries. *J. Cereb. Blood Flow Metab.*, **1**, 339–347.
- VAN BREEMEN, C. (1969). Blockade of membrane calcium fluxes by lanthanum in relation to vascular smooth muscle contractility. *Archs. int. Physiol. Biochem.*, **77**, 710–716.
- VAN BREEMEN, C., FARINAS, B.R., GERBA, P. & McNAUGHTON, E.D. (1972). Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circulation Res.*, **30**, 44–54.

- BRIGGS, A.H. (1962). Calcium movements during potassium contracture in isolated rabbit aortic strips. *Am. J. Physiol.*, **203**, 849–852.
- CHURCH, J. & ZSOTÉR, T.T. (1980). Calcium antagonistic drugs. Mechanism of action. *Can. J. Physiol. Pharmacol.*, **58**, 254–264.
- DETH, R. & VAN BREEMEN, C. (1974). Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release drug induced activation of the rabbit aorta. *Pflügers Arch.*, **348**, 13–22.
- EDVINSSON, L., HARDEBO, J.E., McCULLOCH, J. & OWMAN, C. (1978). Effects of dopaminergic agonists and antagonists on isolated cerebral blood vessels. *Acta physiol. scand.*, **104**, 349–359.
- FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *A. Rev. Pharmac. Tox.*, **17**, 149–166.
- GREENBERG, R.A. & SPARKS, H.V. (1969). Prostaglandins and consecutive vascular segments of the canine hindlimb. *Am. J. Physiol.*, **216**, 567–571.
- HINKE, J.A.M. (1965). Calcium requirements for noradrenaline and high potassium ion contraction in arterial smooth muscle. In *Muscle*, ed. Paul, W., Daniel, E.E., Kay, C.M. & Monckton, G. pp. 269–284. London: Pergamon Press.
- HÖGESTÄTT, E.D., ANDERSSON, K.-E. & EDVINSSON, L. (1983). Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta physiol. scand.*, (in press).
- HUDGINS, P.M. & WEISS, G.B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmac. exp. Ther.*, **159**, 91–97.
- JOHANSSON, B. (1978). Processes involved in vascular smooth muscle constriction and relaxation. *Circulation Res.*, **43**, Suppl. I, I–14–I–20.
- MIKKELSEN, E., ANDERSSON, K.-E. & LEDERBALLE PEDERSEN, O. (1979). Verapamil and nifedipine inhibition of contractions induced by potassium and noradrenaline in human mesenteric arteries and veins. *Acta pharmac. tox.*, **44**, 110–119.
- NAKANO, J. (1968). Effects of prostaglandins E_1 , A_1 and F_{2a} on the coronary and peripheral circulations. *Proc. Soc. exp. Biol. Med.*, **127**, 1160–1163.
- PAYET, M.D., SCHANNE, O.F., RUIZ-CERETTI, E. & DEMERS, J.-M. (1980). Inhibitory activity of blockers of the slow inward current in rat myocardium, a study in steady state and of rate of action. *J. molec. cell. Cardiol.*, **12**, 187–200.
- SHIMIZU, K., OHTA, T. & TODA, N. (1980). Evidence for greater susceptibility of isolated dog cerebral arteries to Ca antagonists than peripheral arteries. *Stroke*, **11**, 261–266.
- TODA, N. (1976). Potassium-induced relaxation in isolated cerebral arteries contracted with prostaglandin F_{2a} . *Pflügers Arch.*, **364**, 235–242.
- TOWART, R. & KAZDA, S. (1980). Selective inhibition of serotonin-induced contractions of rabbit basilar artery by nimodipine (BAY e 9736). *IRCS Med. Sci.*, **8**, 206.
- USKI, T.K., EDVINSSON, L. & OWMAN, C. (1981). Effects of prostaglandin E_1 , E_2 and F_{2a} on isolated pial arteries of cat. *Acta physiol. scand.*, **111**, 487–490.

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**INFLUENCE OF EXTRACELLULAR CALCIUM AND NIFEDIPINE
ON α_1 -AND α_2 -ADRENOCEPTOR-MEDIATED CONTRACTILE RESPONSES
IN ISOLATED RAT AND CAT CEREBRAL AND MESENTERIC ARTERIES**

By

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Running title: Vascular α -adrenoceptor subtypes and calcium

ABSTRACT

T. Skärby, E. D. Högestätt and K.-E. Andersson: Influence of extracellular calcium and nifedipine on α_1 - and α_2 -adrenoceptor mediated contractile responses in isolated rat and cat cerebral and mesenteric arteries.

The influence of extracellular Ca^{2+} and nifedipine on contractile responses to 10 μM noradrenaline (NA) was investigated in isolated rat and cat middle cerebral (RMCA, CMCA) and mesenteric (RMA, CMA) arteries. In the CMCA (containing predominantly α_2 -adrenoceptors), the NA-induced contractions developed considerably slower than in the RMCA, RMA (containing mainly α_1 -adrenoceptors) and CMA (sensitive to both α_1 - and α_2 -adrenoceptor selective antagonists). The tonic component of the NA-induced contraction in the four types of artery was substantially suppressed after only short periods in Ca^{2+} -free solution. The contractile response to 124 mM K^+ in each type of artery, excluding the CMCA, was more sensitive to Ca^{2+} deprivation than was that to NA. This suggests that NA, besides mobilizing extracellular Ca^{2+} , also can release Ca^{2+} from an intracellular pool in the RMCA, RMA and CMA, but not in the CMCA. Thus, α_1 -adrenoceptor-mediated contractions in the RMCA and RMA seem to depend on both Ca^{2+} influx and intracellular Ca^{2+} release, whereas α_2 -adrenoceptor-mediated contractile responses in the CMCA appear to rely almost entirely on Ca^{2+} influx. Both the maximum response and the tonic component of the NA-induced contraction were significantly more sensitive to nifedipine in the CMCA than in the RMCA. In comparison with the NA-induced contractions in these arteries, those in the RMA and CMA were relatively resistant to nifedipine. Notably, the maximum inhibition of the tonic component produced by nifedipine appeared to be inversely related to the amplitude of the contraction elicited by NA in Ca^{2+} -free medium. The nifedipine resistant part of the contractile response to NA in the different arteries was not appreciably influenced (RMA, RMCA) or only slightly reduced (CMA, CMCA) by prior K^+ depolarization. In the CMCA exposed to NA in Ca^{2+} -free medium, nifedipine almost abolished the contraction induced by re-addition of Ca^{2+} , whereas in the other types of artery, Ca^{2+} re-application evoked a significant contraction also in the

presence of the drug. The differential effects of nifedipine presumably reflect differences between the arteries not only in the relative contribution of Ca^{2+} influx and intracellular Ca^{2+} release to the contractile activation, but also in the nifedipine sensitivity of the Ca^{2+} entry pathways utilized by NA. It is concluded that the mechanism through which NA induces contraction seems to be related both to the subtype of α -adrenoceptor stimulated by NA and to the anatomical origin of the vessel.

Key words: Vascular smooth muscle; excitation-contraction coupling; α -adrenoceptors; extracellular calcium; nifedipine

INTRODUCTION

Both α_1 - and α_2 -adrenoceptors have been demonstrated post-junctionally in vascular smooth muscle (VSM); stimulation of either type of receptor leading to contraction (Drew & Whiting 1979, Timmermans et al. 1979, Docherty & McGrath 1980, Skärby et al. 1981, Högestätt & Andersson 1984). Much interest has recently been focused on the mechanisms providing activator Ca^{2+} to the contractile proteins after α_1 - and α_2 -adrenoceptor stimulation. In pithed rats and cats, ganglion-blocked rabbits (van Zwieten et al. 1982) and in the isolated dog hindlimb (Llenas & Massingham 1983), vasopressor responses induced by selective α_1 -adrenoceptor agonists, but not those elicited by selective α_2 -adrenoceptor agonists, were shown to be relatively resistant to organic " Ca^{2+} entry blockers" (Fleckenstein 1977). These observations led to the suggestion that α_1 -adrenoceptors in VSM are coupled to release of intracellularly stored Ca^{2+} , whereas stimulation of α_2 -adrenoceptors promotes the influx of Ca^{2+} from the extracellular medium (see van Meel 1982). The experiments on which this conclusion was based were largely conducted on whole animal preparations. However, studies of isolated blood vessels have resulted in divergent conclusions (DeMey & Vanhoutte 1981, Cauvin et al. 1982, Caverio et al. 1983, Godfraind et al. 1982).

In order to further elucidate the cellular mechanisms coupled to postjunctional α_1 - and α_2 -adrenoceptors and to appreciate regional differences in these mechanisms, we compared the effects of Ca^{2+} deprivation and nifedipine on contractile responses induced by noradrenaline (NA) in isolated rat middle cerebral and mesenteric arteries (containing mainly α_1 -adrenoceptors) and cat middle cerebral (containing predominantly α_2 -adrenocpetors) and mesenteric (sensitive to both α_1 - and α_2 -adrenoceptor selective antagonists) arteries.

MATERIAL AND METHODS

Vascular preparations

Rats (Sprague-Dawley) and barbiturate anaesthetized cats (pentobarbital, 30 mg/kg i.p.) of either sex were decapitated and the brain and part of the mesenterium rapidly removed and put into cold (8 - 12°C) "standard" Krebs solution (for composition, see below). Rat and cat middle cerebral (RMCA, CMCA) and mesenteric (RMA, CMA) arteries were subsequently dissected out under a microscope. The outer diameter of the excised arteries decreased in the following order: CMA (0.5 mm; third generation of branches originating from the superior CMA) > CMCA (0.4 mm) > RMA (0.2 mm; second generation of branches originating from the superior RMA) > RMCA (0.1 mm). The vessels were either used immediately or stored in a refrigerator (8 - 12°C) for use within 24 hours.

Experimental procedure

Ring segments of the arteries (1 - 3 mm long) were suspended between two metal holders (diameter = 50 - 200 µm) in small volume (2.5 - 5 ml) vessel baths. The mechanical activity was recorded "isometrically" by means of Grass Instruments FT03C force-displacement transducers, and the output from the transducers was displayed on a Grass Instruments polygraph. The temperature of the buffer solution in the vessel baths was kept at 37°C. The bath fluid was continuously bubbled with a mixture of O₂ (95 %) and CO₂ (5 %), resulting in a pH of approximately 7.4. During an equilibration period of one hour, the vessels were frequently stretched until a stable resting tension of approximately 2 mN (rat arteries) or 4 mN (cat arteries) was obtained. For further experimental details see Högestätt et al. (1983) and Skärby et al. (1983). The preparations were not accepted for further experiments unless at least two reproducible contractions induced by NA (10 µM) or an isotonic K⁺-Krebs solution (for composition, see below) were first obtained. Responses were considered reproducible when the variation between consecutive contractions was 10 % or less. Propranolol (0.3 µM) was included in the bath solution when the response to NA was examined in order to block β-adrenoceptor-mediated effects of the agonist. The contraction

induced by NA in standard Krebs solution will hereafter be referred to as the control response.

Buffer solutions

1. The standard Krebs solution contained (in mM): NaCl 119, NaHCO₃ 15, KCl 4.6, NaH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 1.5, glucose 11.
2. The isotonic K⁺-Krebs solution (124 mM K⁺) was prepared by substituting all NaCl in the standard Krebs solution with equimolar amounts of KCl.
3. Ca²⁺-free solutions were prepared by substituting all CaCl₂ with 10 µM EGTA.

Drugs

(±)-Noradrenaline HCl (Sigma), rauwolscine HCl (Roth) and cocaine HCl (Merck) were dissolved in 0.9 % NaCl, containing 0.1 mM ascorbic acid. Prazosin (Pfizer) was dissolved in 10 % lactic acid, giving a final prazosin concentration of 1 mM. Nifedipine (0.1 mg/ml) (Bayer) and propranolol HCl (1 mg/ml) (ICI) were provided in ampoules. Nifedipine was kept dark until used to avoid light-induced degradation. All drugs except those supplied in ampoules were prepared as stock solutions and stored at -70° C until used. Fresh dilutions of the drugs were always prepared on the day of experiment in 0.9 % NaCl, containing 0.1 mM ascorbic acid. All concentrations given in "Results" refer to the final bath concentrations.

Statistics

Student's t-test (two-tailed) was used to determine statistical significance and the 0.05 level of probability was accepted as significant. However, the effects of nifedipine were compared by analysis of variance, and in subsequent t-tests, statistical significance was accepted only when $P < 0.01$. The least squares method was used for calculating linear regressions. The results shown in tables, figures and the text are expressed as mean values $\pm$ SEM, with n referring to the number of vessels examined.

RESULTS

Effect of prazosin and rauwolscine on the contractile response to NA

The amplitude of the contractions induced by 10 μ M NA in the RMCA, RMA, CMCA and CMA corresponded to between 90 % and 95 % of that elicited by 0.1 mM NA. When expressed as a percentage of the contractile response to 124 mM K^+ , the contraction induced by 10 μ M NA was 97 ± 4 % (n=9) in the RMA, 109 ± 2 % (n=25) in the CMA, 48 ± 3 % (n=13) in the RMCA and 44 ± 2 % (n=20) in the CMCA.

As can be seen in Figs. 1 and 2, NA (10 μ M) induced an initial rapid tension increase followed by a tonic response in the RMCA, RMA and CMA. In the CMCA, the NA-induced contractions developed considerably slower than in the other types of artery. The contractions usually stabilized after 5 min, and the amplitude of the response at this time will hereafter be referred to as the tonic component. Figs. 1 and 2 also show the effects of prazosin (1 nM - 10 μ M) and rauwolscine (1 nM - 10 μ M) on the contractile responses to NA in the four types of artery. Although the potencies of the α -adrenoceptor blockers differed markedly, prazosin and rauwolscine influenced the time course of the NA-induced contractions similarly in each type of vessel.

Contractile responses to NA and K^+ in Ca^{2+} -free medium

Omission of Ca^{2+} from the bath solution consistently suppressed the contractile response to NA (10 μ M) in all four types of artery. The reduction of the maximum response and the tonic component of the NA-induced contraction after various time periods in Ca^{2+} -free Krebs solution is shown in Fig. 3. As can be seen, the tonic component was substantially reduced by Ca^{2+} deprivation in all four vessel types. The amplitude of the maximum response to 10 μ M NA after 10 min in Ca^{2+} -free medium decreased in the following order between the various vessel segments: CMA $\approx$ RMA > RMCA >> CMCA; the NA-induced contraction in the CMCA was virtually abolished after this treatment. Excluding the CMCA, in which the responses to K^+ and NA were equally reduced by a 10-min preincubation in Ca^{2+} -free medium, the K^+ -induced contraction was significantly more depressed by Ca^{2+} deprivation than was the response to NA (Fig. 4).

Effects of nifedipine on K^+ - and NA-induced contractions

Nifedipine effectively relaxed K^+ -induced contractions in the RMCA and CMCA (Fig. 5). Neither the maximum relaxation nor the concentration producing half maximum relaxation differed significantly between the two arteries (Table 1). The contractile response to NA (10 μ M), on the other hand, was more sensitive to nifedipine in the CMCA than in the RMCA. This was true both for the maximum response and for the tonic component of the NA-induced contraction (Fig. 6, Table 2). In contrast to the NA-induced contractions in the RMCA and CMCA, those in the RMA and CMA were relatively resistant to nifedipine (Fig. 6, Table 2). Furthermore, in the RMCA and RMA, but not in the CMCA and CMA, the tonic component of the contractile response to NA was more inhibited by nifedipine than was the maximum response (Fig. 6a, Table 2). This difference was particularly apparent in the RMCA, which in the presence of 0.3 μ M nifedipine responded to NA with a rapid phasic contraction followed by a tonic response of lower amplitude. Interestingly, nifedipine was approximately equipotent in inhibiting NA-induced contractions and in relaxing contractions evoked by K^+ in the CMCA. However, in the RMCA, the contractile response to K^+ was slightly more sensitive to nifedipine than either the maximum response or the tonic component of the NA-induced contraction.

On the basis of the maximum contractile response elicited by 10 μ M NA, the arteries could be ranked in the following order with respect to their ability to contract in the presence of nifedipine: RMA $\approx$ CMA $\gg$ RMCA $\gg$ CMCA. As demonstrated by regression analysis, a close correlation ($r=0.99$; $P < 0.01$) was found between the degree to which 3 μ M nifedipine inhibited the tonic component and the maximum amplitude of the NA-induced contraction after 10 min in Ca^{2+} -free medium (Fig 7). The inhibition produced by nifedipine in the different arteries was reversible, as frequent washes in drug-free solution restored the NA response.

Contractile response to NA in K^+ -depolarized muscle

The isotonic K^+ - Krebs solution evoked strong and sustained contractions in all four types of artery. However,

preparations preincubated with 0.3 μM nifedipine responded to K^+ with a transient contraction, which stabilized after 5 to 10 min at a level slightly above the baseline tension. As shown in Fig. 8, the nifedipine resistant part of the NA-induced contractions was unaltered (RMCA and RMA) or only slightly reduced (CMCA and CMA) by prior K^+ -depolarization; the maximum response to NA in K^+ -depolarized preparations was $98 \pm 5\%$ ($n=6$) in the RMCA, $103 \pm 4\%$ ($n=8$) in the RMA, $69 \pm 2\%$ ($n=6$) in the CMCA and $87 \pm 3\%$ ($n=6$) in the CMA, as compared with that elicited by NA before application of K^+ (also obtained in the presence of nifedipine).

Effect of nifedipine on Ca^{2+} -induced contractions in NA-exposed arteries

In a separate series of experiments, all four types of artery were repeatedly exposed to NA (10 μM) in Ca^{2+} -free Krebs solution until the NA response was reduced to a minimum; a small sustained contraction, less than 5 % of the control response, sometimes persisted. Reapplication of Ca^{2+} (1.5 mM) to the RMA and CMA, exposed to NA, induced a rapid and sustained contraction (Fig. 9). The amplitudes of the Ca^{2+} -evoked contractions in these arteries amounted to $98 \pm 1\%$ ($n=6$) and $90 \pm 2\%$ ($n=6$) of the control response. If nifedipine (0.3 μM) was introduced into the bath solution 15 to 20 min before Ca^{2+} , it suppressed the Ca^{2+} -induced contractions by $33 \pm 4\%$ ($n=6$) and $67 \pm 1\%$ ($n=6$) in the RMA and CMA, respectively. Nifedipine also delayed the onset of the contractile response to Ca^{2+} by 15 to 45 seconds and made the tension increase less rapidly.

The contractile responses to 1.5 mM Ca^{2+} in the " Ca^{2+} -deprived" RMCA and CMCA exposed to NA were more complex and less reproducible than those obtained in the RMA and CMA. As shown in Fig. 9, the Ca^{2+} -induced contractions were composed of an initial phasic response followed by a tonic component. The peak tension of the initial transient contraction, relative to the control response, was $65 \pm 15\%$ ($n=6$) in the RMCA and $46 \pm 12\%$ ($n=6$) in the CMCA. In the RMCA, the tonic component of the Ca^{2+} -induced contraction usually required 10 to 20 min to be fully developed (not shown). A similar slow tension increase was not observed in the CMCA. Nifedipine (0.3 μM) almost abolished the Ca^{2+} -induced

contraction in the CMCA (n=4). However, in the RMCA, a significant portion of the response still remained in the presence of the drug. The amplitude of this residual contraction amounted to $41 \pm 4\%$ (n=6) of the control response (elicited by NA in standard Krebs solution).

DISCUSSION

The postjunctional α -adrenoceptors in the RMCA, RMA, CMCA and CMA have recently been characterized, by means of subtype selective α -adrenoceptor agonists and antagonists. Thus, it was suggested that the postjunctional α -adrenoceptors in the RMCA and RMA are mainly of the α_1 -type (Högestätt & Andersson 1984). Using radioligand binding technique, Colucci et al. (1980, 1981) came to a similar conclusion regarding the α -adrenoceptor population in rat mesenteric arteries. Skärby et al. (1981, 1983) provided evidence suggesting that the α -adrenoceptors in the CMCA are predominantly of the α_2 -type, and this view was supported by the findings of Medgett and Langer (1983). The CMA, on the other hand, was suggested to contain a "broader" type of α -adrenoceptor, unable to differentiate between antagonists that in other tissues act preferentially on α_1 - or α_2 -adrenoceptors (Skärby et al. 1983). Since each of the arteries examined in the present study was supplied with a relatively homogenous population of α -adrenoceptors, we could use the non-selective agonist NA to rather selectively stimulate either α_1 - or α_2 -adrenoceptors on the VSM cells.

Components of the NA-induced contraction

The contractile response to NA in the RMCA, RMA and CMA could usually be divided into an initial fast and an ensuing tonic component (cf. Bohr 1963, Watkins & Davidson 1980). However, in the CMCA, NA produced mainly a monophasic slow tension increase. In the pithed rat preparation, Timmermans & van Zwieten (1980) and van Meel et al. (1981) described that the pressor response elicited by selective α_2 -adrenoceptor agonists developed more slowly than that induced by selective α_1 -adrenoceptor agonists. From these observations, it was speculated that the fast component and the slow component of the contractile response induced by nonselective α -adrenoceptor agonists in the isolated rabbit aorta were caused by stimulation of α_1 - and α_2 -adrenoceptors, respectively (van Meel et al. 1981). This implies that the two contraction components should be affected differently by selective α -adrenoceptor blockers. As shown in the present study, prazosin and rauwolscine affected the time course of the NA-induced contraction similarly in each type of artery. Furthermore, even

though the postjunctional α -adrenoceptors in the rabbit aorta, RMCA and RMA have been classified as being predominantly of the α_1 -type (Docherty et al. 1981, Högestätt & Andersson 1984), the contractions induced by NA could still be resolved into two components in these vessels. These findings do not favour the view that the two components of the contractile response to NA are attributable to stimulation of different α -adrenoceptor subtypes. Nevertheless, the rate of tension development induced by NA was considerably slower in the CMCA (containing predominantly α_2 -adrenoceptors) than in the RMCA and RMA (mainly endowed with α_1 -adrenoceptors), suggesting differences in the excitation-contraction coupling process associated with stimulation of the two receptor subtypes.

Ca²⁺ pools used for activation

The fast and slow components of NA-induced contractions seen in several VSM preparations have been attributed to mobilization of different Ca²⁺ pools (Steinsland et al. 1973, Deth & van Breemen 1974, Bolton 1979). As shown in the different arteries presently studied, the main part of the tonic component of the contractile response to NA was abolished after only short incubation periods in Ca²⁺-free solution. This suggests that the Ca²⁺ utilized for contraction during the tonic component is derived mainly from the extracellular medium, irrespective of the α -adrenoceptor subtype stimulated by NA.

High K⁺ solutions are generally believed to induce VSM contraction by enhancing Ca²⁺ entry from the extracellular medium (Bolton 1979). The observation that the contractile response to K⁺ in the RMCA, RMA and CMA was reduced to a larger extent by Ca²⁺ deprivation than was that elicited by NA argues that NA, in addition to mobilizing extracellular Ca²⁺, also can liberate Ca²⁺ from a more tightly bound pool (hereafter referred to as intracellular Ca²⁺) in these arteries. These results do not support the view that α_1 -adrenoceptors are linked specifically to either of these processes. A similar conclusion was reached by Cauvin et al. regarding the α_1 -adrenoceptors in the rabbit aorta (1982). However, in the CMCA, K⁺- and NA-induced contractions were equally suppressed and almost abolished after the same treatment

(present study), suggesting that the contractile response to NA in this preparation rely almost entirely on influx of Ca^{2+} from the extracellular medium. The failure of NA to liberate intracellularly stored Ca^{2+} in the CMCA might reflect a general lack of coupling between α_2 -adrenoceptors and intracellular Ca^{2+} release, as suggested by, e.g., van Meel (1982).

As judged from the amplitude of the contractile response to NA in Ca^{2+} -free medium, the amount of intracellular Ca^{2+} released by NA appeared to be smaller in the RMCA than in the CMA and RMA, and almost negligible in the CMCA. In addition, the degree of intracellular Ca^{2+} release does not seem to be related to the calibre of the vessel, but rather to the region from which the artery was excised, as NA-induced contractions in mesenteric arteries were always more resistant to Ca^{2+} deprivation than were those in cerebral arteries. Evidence of regional differences in the excitation-contraction coupling process between cerebral and peripheral arteries has previously been reported for both animal and man (Allen & Banghart 1979, Shimizu et al. 1980, Brandt et al. 1981, McCalden and Bevan 1981, Towart 1981, Bevan 1983, Yamamoto et al. 1983).

Differential effects of nifedipine on NA- and K^+ -induced contractions

Analogous with its mechanism of action on heart muscle, the relaxing effect of nifedipine on VSM has been explained by inhibition of Ca^{2+} entry (Fleckenstein 1977, Andersson & Högestätt 1984). Although Ca^{2+} entry blockers, such as nifedipine, verapamil and diltiazem, are commonly considered to be relatively selective inhibitors of Ca^{2+} influx through potential-operated Ca^{2+} channels (POCs) (Bolton 1979, Andersson & Högestätt 1984), recent studies suggest that receptor-operated Ca^{2+} channels (ROCs) in certain VSM preparations may also be effectively blocked by these drugs (Walus et al. 1981, Cauvin et al. 1982, Bevan 1983).

In the present study, contractions elicited by NA in the RMA and CMA were relatively resistant to nifedipine. Although the tonic component of the NA-induced contraction in these vessels was substantially suppressed by Ca^{2+} deprivation, $0.3 \mu\text{M}$ nifedipine (a concentration producing a maximum relaxation of K^+ -

induced contractions in the RMCA and CMCA) only reduced the tonic response to NA by approximately 30 %. Moreover, the nifedipine-resistant part of the NA-induced contraction appeared to be relatively independent of the state of polarization of the cell membrane, as it was unaffected (RMA) or only slightly reduced (CMA) by prior K^+ -depolarization. Reintroduction of Ca^{2+} to the RMA and CMA, which had been depleted of Ca^{2+} and exposed to NA, induced sustained and reproducible contractions of approximately the same magnitude as those elicited by NA in standard Krebs solution. Although the Ca^{2+} -induced contractions may be regarded as reflecting Ca^{2+} influx only, nifedipine (0.3 μM) still failed to abolish the contractile response to Ca^{2+} in the RMA and CMA. These results are compatible with the view that NA can activate a population of ROCs resistant to nifedipine in these arteries.

Nifedipine effectively relaxed the K^+ -contracted RMCA and CMCA, and the relaxation produced did not differ significantly between the two arteries. However, the NA-induced contraction (both the maximum response and the tonic component) was more sensitive to nifedipine in the CMCA than in the RMCA. Pronounced regional differences were also observed, as the maximum response and tonic component of the NA-induced contraction in the RMCA and CMCA were considerably more inhibited by nifedipine than were those in the corresponding mesenteric arteries with respect to both the I_{max} and IC_{50} values. Considering that the size of the RMA and RMCA was smaller than that of the CMCA, these findings do not agree with the observations by Godfraind and co-workers suggesting, that the degree of reduction of the contractile response to NA by the putative Ca^{2+} entry blockers flunarizine and cinnarizine was inversely related to the calibre of the vessel (Broekaert & Godfraind 1979, Godfraind & Dieu 1981).

Interestingly, NA- and K^+ -induced contractions in the CMCA were similarly sensitive to both nifedipine and Ca^{2+} removal. In addition, nifedipine almost abolished the contraction induced by Ca^{2+} re-addition in arteries exposed to NA in Ca^{2+} -free medium. These findings may indicate that K^+ and NA largely utilize the same pathway to increase the myoplasmic Ca^{2+} concentration. Electrophysiological studies on isolated cat

cerebral arteries have shown a close correlation between NA-induced contraction and membrane depolarization (Harder et al. 1981, Harder 1983). It therefore seems possible that nifedipine inhibited the contractile response to NA in the CMCA by blocking POCs, activated by NA-induced membrane depolarization.

In the RMCA, the maximum response of the NA-induced contraction was less inhibited by nifedipine than the tonic component. Release of intracellularly stored Ca^{2+} during the initial fast component of the NA-induced contraction, can probably explain this difference. The tonic component of the contractile response to NA was only slightly less sensitive to nifedipine than was the K^{+} -induced contraction. Although electrophysiological evidence is lacking, it may be speculated that part of the tonic component of the NA-induced contraction in the RMCA was caused by membrane depolarization and subsequent influx of Ca^{2+} through POCs. However, three pieces of evidence indicate the existence of a nifedipine resistant Ca^{2+} entry pathway, possibly equivalent to a ROC, in the RMCA. First, nifedipine failed to abolish the tonic component of the contractile response to NA. Secondly, the nifedipine resistant part of the NA-induced contraction was present also after prior K^{+} depolarization. Thirdly, the contraction induced by reintroduction of Ca^{2+} to the Ca^{2+} -depleted RMCA was not abolished by nifedipine.

The sensitivity of the NA-induced contraction (both the maximum response and tonic component) to nifedipine decreased in the following order between the different arteries: CMCA >> RMCA >> CMA $\approx$ RMA. This differential effect of nifedipine may reflect differences between the vessels not only in the extent of intracellular Ca^{2+} release, but also in the nifedipine sensitivity of the Ca^{2+} entry pathways utilized by NA. Interestingly, the maximum inhibition of the tonic component produced by nifedipine appeared to be correlated to the amplitude of the contraction elicited by NA in Ca^{2+} -free medium. This suggests that the nifedipine resistant part of the tonic component somehow is related to the extent to which NA releases intracellular Ca^{2+} in that vessel, i.e. the greater the intracellular Ca^{2+} release, the greater the nifedipine resistance of the tonic response. Such a correlation would be anticipated if

the nifedipine resistant part of the tonic component reflects a fast refilling of the NA sensitive intracellular Ca^{2+} store directly from the extracellular medium, and subsequent release of Ca^{2+} into the myoplasm. Casteels and Droogmans (1981) have previously suggested that such a pathway, resistant to Ca^{2+} entry blockers, may represent an alternative model of the ROC.

In conclusion, the results of the present study suggest that stimulation of vascular α_1 -adrenoceptors in the RMCA and RMA can induce both Ca^{2+} influx and intracellular Ca^{2+} release, whereas activation of postjunctional α_2 -adrenoceptors in the CMCA appears to stimulate Ca^{2+} influx only. The presence of different α -adrenoceptor subtypes cannot by itself adequately explain the differences found between cerebral and mesenteric arteries in the sensitivity of the NA-induced contraction to Ca^{2+} deprivation and nifedipine. It is suggested that the mechanism through which NA induces contractions is related not only to the subtype of α -adrenoceptor stimulated by NA, but also to the anatomical origin of the vessel.

REFERENCES

- ALLEN, G.S. & BANGHART, S.B. 1979. Cerebral arterial spasm: Part 9. In vitro effects of nifedipine on serotonin-, phenylephrine- and potassium-induced contractions of canine basilar and femoral arteries. *Neurosurg* 4:37-42.
- ANDERSSON, K.-E., & HÖGESTÄTT, E.D. 1984. On the mechanism of action of calcium antagonists. *Acta Med Scand Suppl.* 681:11-24, 1984.
- BEVAN, J.A. 1983. Diltizem selectively inhibits cerebrovascular extrinsic but not intrinsic myogenic tone. *Circ Res Suppl.* 52:104-109.
- BOHR, D.F. 1963. Vascular smooth muscle: dual effect of calcium. *Science* 139:579-599.
- BOLTON, T.B. 1979. Mechanisms of action of transmitters and other substances on smooth muscle. *Pharmacol Rev* 59:606-718, 1979.
- BRANDT, L., ANDERSSON, K.-E., EDVINSSON, L. & LJUNGGREN, B. 1981. Effects of extracellular calcium and of calcium antagonists on the contractile responses of isolated human pial and mesenteric arteries. *J Cerebral Blood Flow Metabol* 1:339-347.
- BROEKAERT, A. & GODFRAIND, T. 1979. A comparison of the inhibitory effect of cinnarizine and papaverine on the noradrenaline- and calcium-evoked contraction of isolated rabbit aorta and mesenteric arteries. *Eur J Pharmacol* 53:281-288.
- CASTEELS, R., & DROOGMANS, G. 1981. Exchange characteristics of the noradrenaline sensitive calcium store in vascular smooth muscle cells of rabbit ear artery. *J Physiol (Lond.)* 317:263-279.
- CAUVIN, C., LOUTZENHISER, R., HWANG, O. & VAN BREEMEN, C. 1982. α_1 -adrenoceptors induce Ca^{2+} influx and intracellular Ca^{2+} release in isolated rabbit aorta. *Eur J Pharmacol* 84:233-235.

- CAUVIN, C., SAIDA, K. & VAN BREEMEN, C. 1982. Effects of Ca^{2+} antagonists on Ca^{2+} fluxes in resistance vessels. *J Cardiovasc Pharmacol Suppl.* 4:287-290.
- CAVERO, I., SHEPPERSON, N., LEFEVRE-BORG, F. & LANGER, S.Z. 1983. Differential inhibition of vascular smooth muscle responses to α_1 - and α_2 -adrenoceptor agonists by diltiazem and verapamil. *Circ Res Suppl.* 1, 52:69-76.
- COLUCCI, W.S., GIMBRONE, Jr., M.A. & ALEXANDER, R.W. 1980. Characterization of postsynaptic α -adrenergic receptors by ^3H -dihydroergocryptine binding in muscular arteries from the rat mesentery. *Hypertension* 2:149-155.
- COLUCCI, W.S., GIMBRONE, Jr., M.A. & ALEXANDER, R.W. 1981. Regulation of the postsynaptic α -adrenergic receptor in rat mesenteric artery. Effects of chemical sympathectomy and epinephrine treatment. *Circ Res* 48:104-111.
- DE MEY, J. & VANOUTTE, P. 1981. Uneven distribution of post-junctional α_1 - and α_2 -adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 48:875-884.
- DETH, R. & VAN BREEMEN, C. 1974. Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta. *Pflügers Arch* 348:13-22.
- DOCHERTY, J.R., CONSTANTINE, J.W. & STARKE, K. 1981. Smooth muscle of rabbit aorta contains α_1 - but not α_2 -adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 317:5-7.
- DOCHERTY, J.R. & MCGRATH, J.C. 1980. A comparison of pre- and post-junctional potencies of several α -adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. Evidence for two types of postjunctional α -adrenoceptor. *Naunyn-Schmiedeberg's Arch Pharmacol* 312:107-116.

- DREW, G.M. & WHITING, S.B. 1979. Evidence for two distinct types of postsynaptic α -adrenoceptor in vascular smooth muscle in vivo. Br J. Pharmacol 67:207-215.
- FLECKENSTEIN, A. 1977. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann Rev Pharmacol Toxicol 17:149-166.
- GODFRAIND, T. & DIEU, D. 1981. The inhibition by flunarizine of the norepinephrine-evoked contraction and calcium influx in rat aorta and mesenteric arteries. J Pharmacol Exp Ther 217:510-515.
- GODFRAIND, T., MILLER, R.C. & LIMA, J.S. 1982. Selective α_1 - and α_2 -adrenoceptor agonist-induced contractions and ^{45}Ca fluxes in the rat isolated aorta. Br J Pharmacol 77:597-604.
- HARDER, D.R., ABEL, P.W. & HERMSMEYER, K. 1981. Membrane electrical mechanisms of basilar artery constriction and pial artery dilation by norepinephrine. Circ Res 49:1237-1242.
- HARDER, D.R. 1983. Heterogeneity of membrane properties in vascular muscle cells from various vascular beds. Fed Proc 42:253-256.
- HÖGESTÄTT, E.H. & ANDERSSON, K.-E. 1984. On the postjunctional α -adrenoceptors in rat cerebral and mesenteric arteries. (Submitted).
- HÖGESTÄTT, E.D., ANDERSSON, K.-E. & EDVINSSON, L. 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol Scand 117:49-61.
- LLENAS, J., MASSINGHAM, R. 1983. A comparison of the effects of verapamil and cinnarizine, upon responses elicited by selected α_1 - and α_2 -adrenoceptor agonists in the autoperfused canine hindlimb. Eur J Pharmacol 87:53-59.
- MCCALDEN, T.A. & BEVAN, J.A. 1981. Sources of activator calcium in rabbit basilar artery. Am J Physiol 241:H129-133.

- MEDGETT, I.C. & LANGER, S.Z. 1983. Characterization of smooth muscle α -adrenoceptors and of responses to electrical stimulation in the cat isolated perfused middle cerebral artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 323:24-32.
- SHIMIZU, K., OHTA, T. & TODA, N. 1980. Evidence for greater susceptibility of isolated dog cerebral arteries to Ca antagonists than peripheral arteries. *Stroke* 11:261-265.
- SKÄRBY, T., ANDERSSON, K.-E. & EDVINSSON, L. 1981. Characterization of the postsynaptic α -adrenoceptor in isolated feline cerebral arteries. *Acta Physiol Scand* 112:105-107.
- SKÄRBY, T.V.C., ANDERSSON, K.-E. & EDVINSSON, L. 1983. Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand* 117:63-73.
- STEINSLAND, O.S., FURCHGOTT, R.F. & KIRPEKAR, S.M. 1973. Biphasic vasoconstriction of the rabbit ear artery. *Circ Res* 32:49-58.
- TIMMERMANS, P.B.M.W.M., KWA, H.Y. & VAN ZWIETEN, P.A. 1979. Possible subdivision of postsynaptic α -adrenoceptors mediating pressor responses in the pithed rat. *Naunyn-Schmiedeberg's Arch Pharmacol* 310:189-193.
- TIMMERMANS, P.B.M.W.M., & VAN ZWIETEN, P.A. 1980. Vasoconstriction mediated by postsynaptic α_2 -adrenoceptor stimulation. *Naunyn-Schmiedeberg's Arch Pharmacol* 313:17-20.
- TOWART, R. 1981. The selective inhibition of serotonin-induced contractions of rabbit cerebral vascular smooth muscle by calcium-antagonistic dihydropyridines. An investigation of the mechanisms of actions of nimodipine. *Circ Res* 48:650-657.
- VAN MEEL, J.C.A. 1982. The vascular postjunctional α_2 -adrenoceptor. Thesis, University of Amsterdam.

- VAN MEEL, J.C.A. DE JONGE, A., KALKMAN, H.O., WILFFERT, B.,
TIMMERMANS, P.B.M.W.M., & VAN ZWIETEN, P.A. 1981. Organic and
inorganic calcium antagonists reduce vasoconstriction in vivo
mediated by postsynaptic α_2 -adrenoceptors. Naunyn-Schmiedeberg's
Arch Pharmacol 316:288-293.
- VAN ZWIETEN, P.A., VAN MEEL, J.C.A. & TIMMERMANS, P.B.M.W.M. 1982.
Calcium antagonists and α_2 -adrenoceptors: possible role of
extracellular calcium ions in α_2 -adrenoceptor-mediated
vasoconstriction. J Cardiovasc Pharmacol 4:273-279.
- WALUS, K.M., FONDACARO, J.D. & JACOBSON, E.D. 1981. Effects of
calcium and its antagonists on the canine mesenteric circulation.
Circ Res 48:692-700.
- WATKINS, R. W. & Davidson, I.W.F. 1980. Contraction velocity
analysis of norepinephrine and angiotensin-II activation of
vascular smooth muscle. Eur J Pharmacol 62:177-189.
- YAMAMOTO, M., Ohta, T. & TODA, N. 1983. Mechanisms of relaxant action
of nicardipine, a new Ca^{++} -antagonist, on isolated dog cerebral
and mesenteric arteries. Stroke 14:270-275.

Table 1. Relaxation produced by nifedipine of K^+ -contracted rat (RMCA) and cat (CMCA) middle cerebral arteries.

Artery	n	Relaxation	
		$-\log RC_{50}^a$	$R_{max} (\%)^b$
RMCA	7	8.41 ± 0.06	84 ± 2
CMCA	6	8.48 ± 0.05	87 ± 2

^a Logarithm of the nifedipine concentration (M) producing half maximum relaxation

^b Maximum relaxation

Table 2. Inhibition by nifedipine of the maximum response and the tonic component of the contractile response to 10 μ M noradrenaline in rat and cat middle cerebral (RMCA, CMCA) and mesenteric (RMA, CMA) arteries. Conditions as in fig. 6.

Artery	n	I _{max} (%) ^a		-log IC ₅₀ ^b	
		Maximum response to NA	Tonic component of the NA response ^c	Maximum response to NA	Tonic component of the NA response ^c
RMCA	9	48 ± 5	77 ± 3	8.02 ± 0.06	8.02 ± 0.09
RMA	8	25 ± 4	41 ± 6	7.38 ± 0.13	7.15 ± 0.10
CMCA	6	86 ± 2	92 ± 2	8.65 ± 0.11	8.70 ± 0.13
CMA	6	34 ± 3	37 ± 3	7.57 ± 0.07	7.58 ± 0.08

^a Maximum inhibition produced by nifedipine.

^b logarithm of the nifedipine concentration (M) producing half maximum inhibition.

^c The tonic component was defined as the amplitude of the contraction 5 min after application of NA.

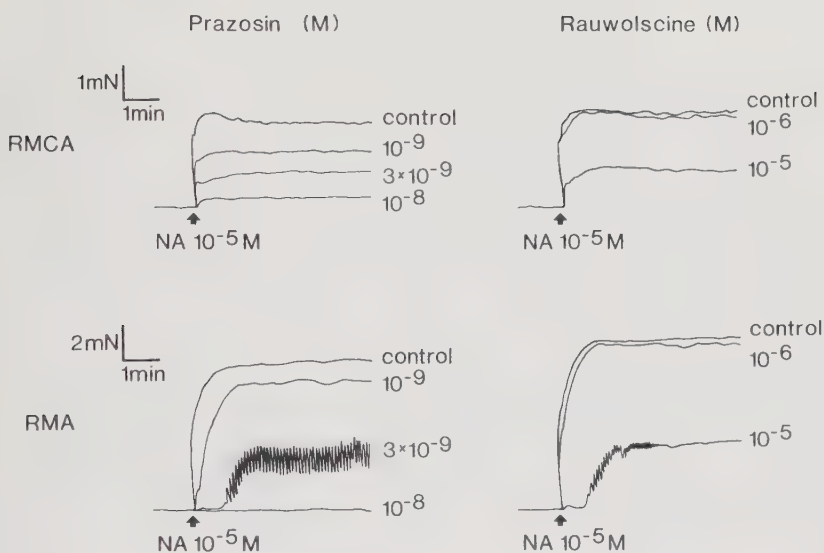


Fig. 1. Superimposed tracings of contractions induced by noradrenaline (NA) in the presence of increasing concentrations of prazosin or rauwolscine in rat middle cerebral (RMCA) and mesenteric (RMA) arteries. The exposure time for each concentration of α -adrenoceptor antagonist was 15 to 20 min. Each series of tension curves was recorded from a separate arterial segment.

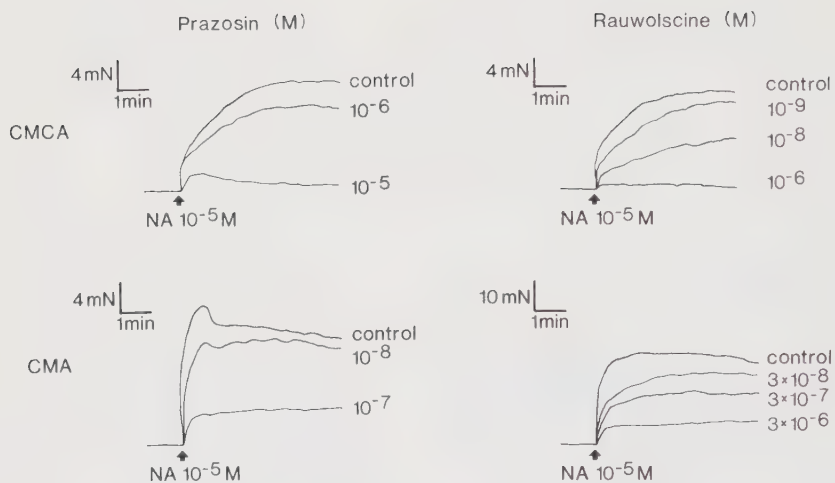


Fig. 2. Suppression of noradrenaline (NA)-induced contractions by increasing concentrations of prazosin and rauwolscine in cat middle cerebral (CMCA) and mesenteric (CMA) arteries. Experimental conditions as in Fig. 1.

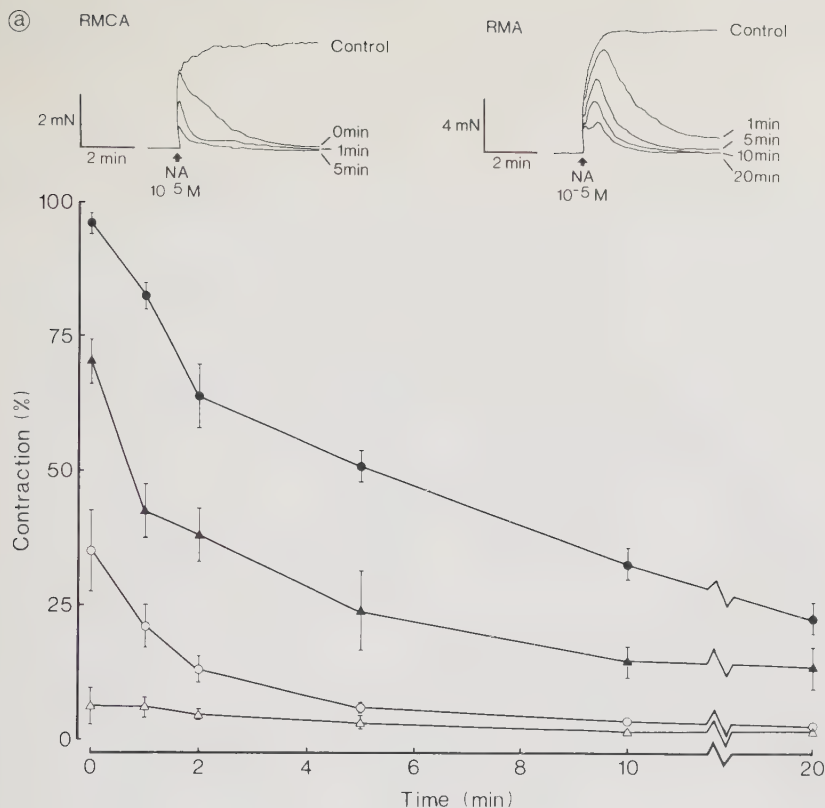


Fig. 3a. Effect of Ca^{2+} removal on the contractile response to $10 \mu\text{M}$ noradrenaline (NA) in rat middle cerebral (RMCA; $\blacktriangle, \triangle$) ($n=5-8$) and mesenteric (RMA; $\bullet, o$) ($n=6-8$) arteries. The time indicated refers to the time period in Ca^{2+} -free medium before application of NA. In some experiments the RMCA and RMA were exposed to a Ca^{2+} -free NA solution without prior treatment in Ca^{2+} -free medium (shown at time "0 min"). The effects of Ca^{2+} omission on the maximum response (filled symbols) and on the tonic component (open symbols) of the NA-induced contraction were evaluated separately and expressed as a percentage of respective response in standard Krebs solution. The tonic component was defined as the amplitude of the contraction 5 min after application of NA. The arteries were allowed to recover in standard Krebs solution for at least 20 min following each exposure to Ca^{2+} -free medium. Upper section: Superimposed tension tracings from two individual arterial segments.

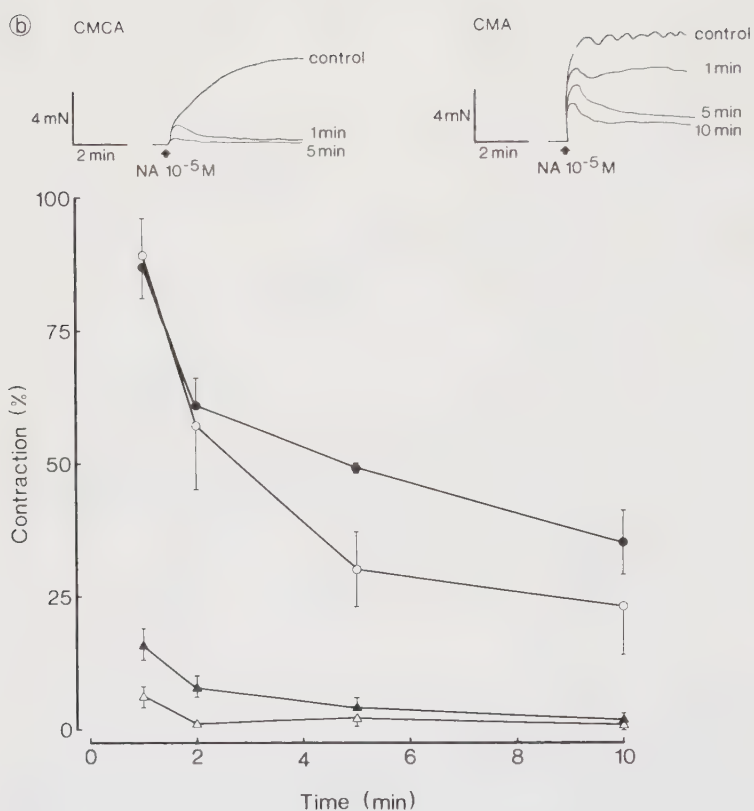


Fig. 3b. Effect of Ca^{2+} removal on the contractile response to noradrenaline (NA) in cat middle cerebral (CMCA; ▲, △) ($n=6$) and mesenteric (CMA; ●, ○) ($n=6$) arteries. For further explanations, see legend to Fig. 3a.

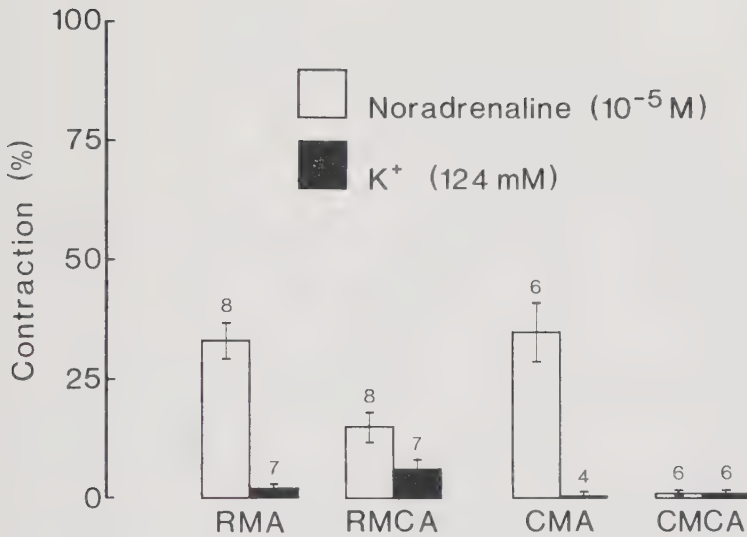


Fig. 4. Reduction of the maximum contractile responses to $10 \mu\text{M}$ noradrenaline (NA) and 124 mM K^{+} following 10 min in Ca^{2+} -free medium. 100 % denotes the maximum contraction evoked by respective stimulant in standard Krebs solution. RMA, rat mesenteric artery; CMA, cat mesenteric artery; RMCA, rat middle cerebral artery; CMCA, cat middle cerebral artery. Figures above the bars indicate the number of vessels examined.

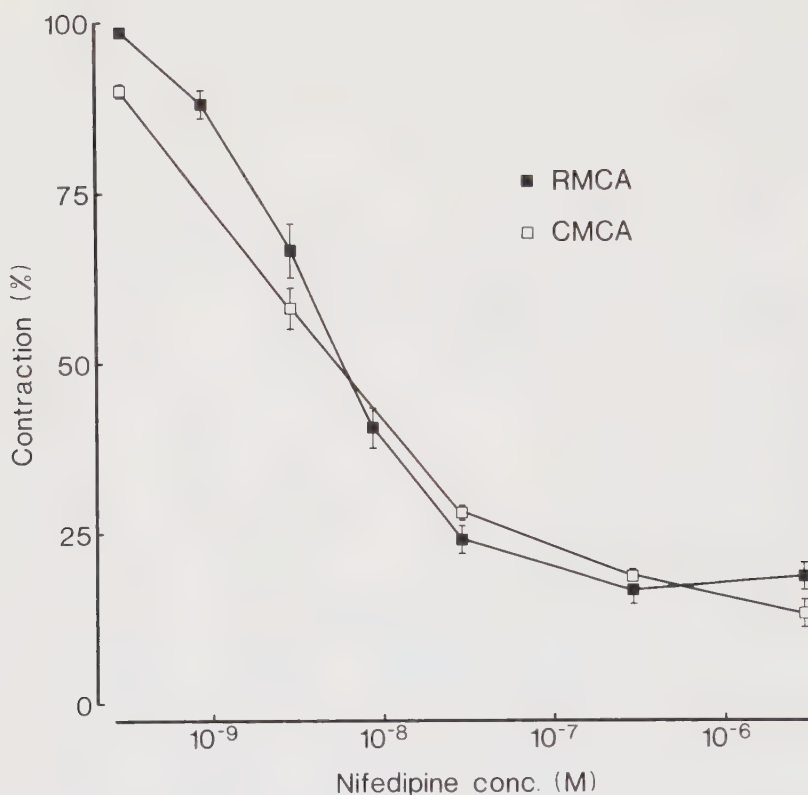


Fig. 5. Concentration-relaxation curves for nifedipine in K^+ -contracted rat (RMCA) ($n=7$) and cat (CMCA) ($n=6$) middle cerebral arteries. Contractions were induced by a high K^+ solution, containing 124 mM K^+ . After approximately 5 min in this medium, at a time when the tension had stabilized, nifedipine was added cumulatively. The tension was allowed to reach a plateau following each application of nifedipine; this usually required between 5 and 10 min.

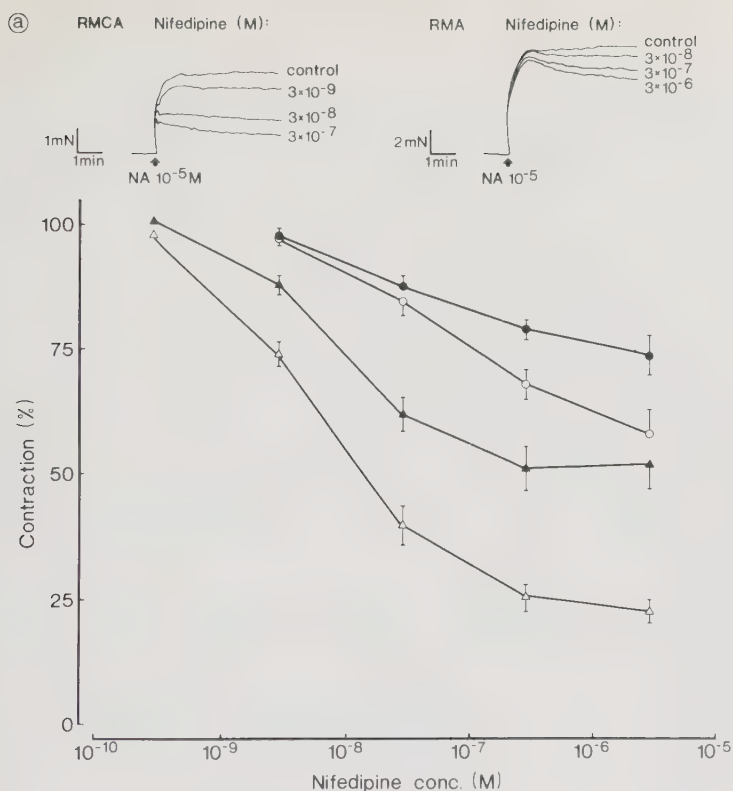


Fig. 6a. Inhibition of noradrenaline (NA)-induced contractions by increasing concentrations of nifedipine in rat middle cerebral (RMCA; ▲, △) ($n=7-9$) and mesenteric (RMA; ●, ○) ($n=6-9$) arteries. Each concentration of nifedipine was applied to the tissue 15 to 20 min before application of NA ($10 \mu\text{M}$). The inhibition produced by nifedipine was calculated in two ways: Filled symbols refer to the maximum response; open symbols refer to the amplitude of the contraction 5 min after application of NA (tonic component). 100 % denotes the maximum response or the tonic component of the NA-induced contraction before introduction of nifedipine. Upper section: Superimposed tension tracings from two individual arterial segments.

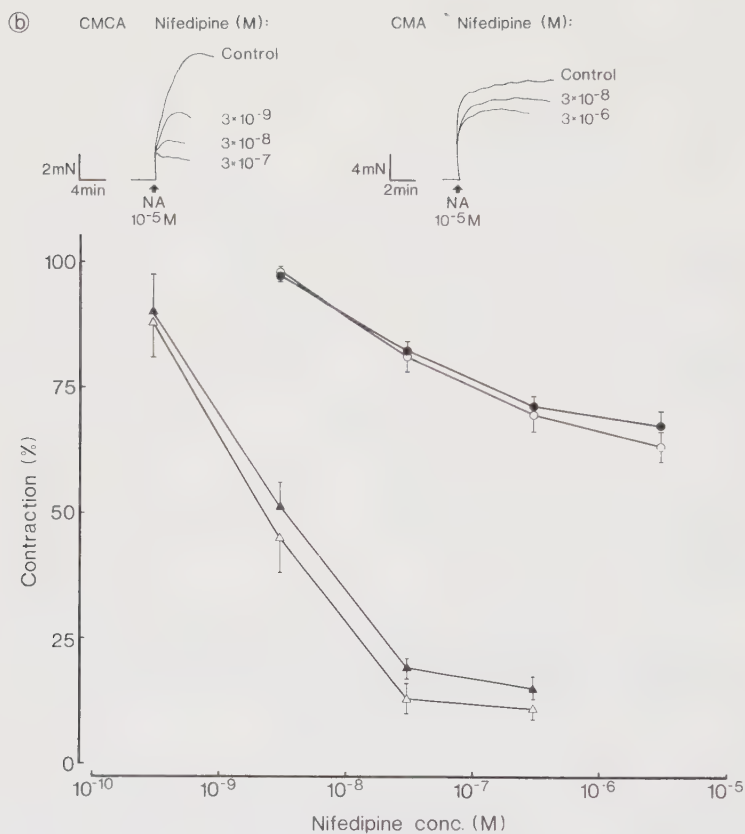


Fig. 6b. Inhibition of noradrenaline (NA)-induced contractions by increasing concentrations of nifedipine in cat middle cerebral (CMCA; ▲, △) ($n=5-7$) and mesenteric (CMA; ●, ○) ($n=7-8$) arteries. For further explanations, see legend to Fig. 6a.

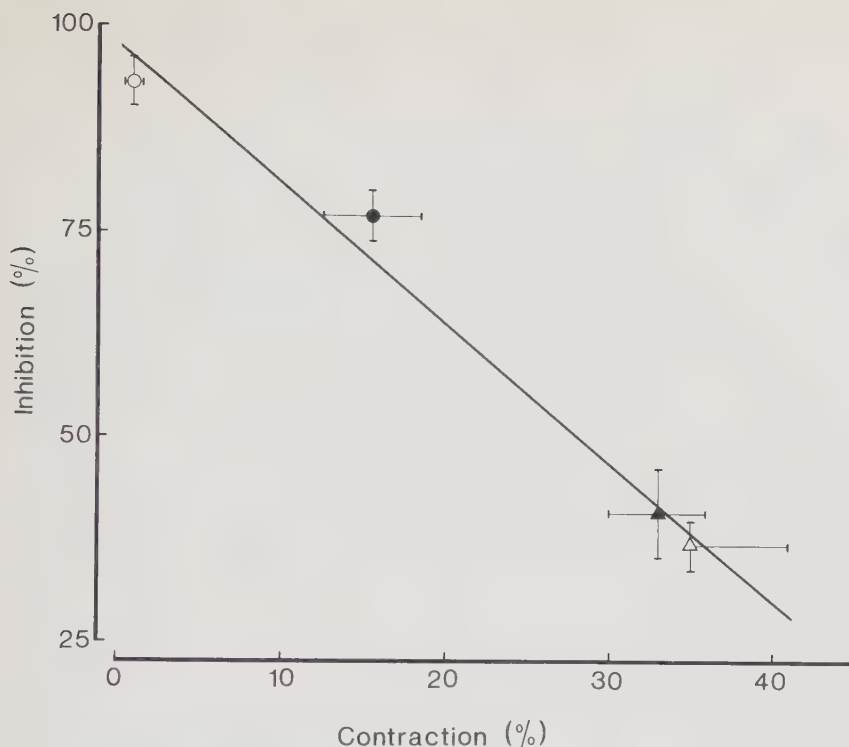


Fig. 7. Linear correlation ($r=-0.99$, $P < 0.01$) between the mean inhibition (ordinate) produced by $3 \mu\text{M}$ nifedipine of the tonic component of the contractile response to $10 \mu\text{M}$ noradrenaline (NA), and the mean amplitude of the contraction (abscissa) elicited by NA after 10 min in Ca^{2+} -free medium, as revealed by regression analysis. ●, rat middle cerebral artery; ▲, rat mesenteric artery; ○, cat middle cerebral artery; △, cat mesenteric artery. The tonic component of the NA-induced contraction was taken as the amplitude of the response 5 min after application of the agonist. The contraction induced by NA in Ca^{2+} -free medium was expressed as per cent of the corresponding control response in standard Krebs solution. Each mean value was based on 5 to 9 experiments. The time of exposure to nifedipine was approximately 15 min. The equation of the regression line is: $y = -1.7x + 98$.

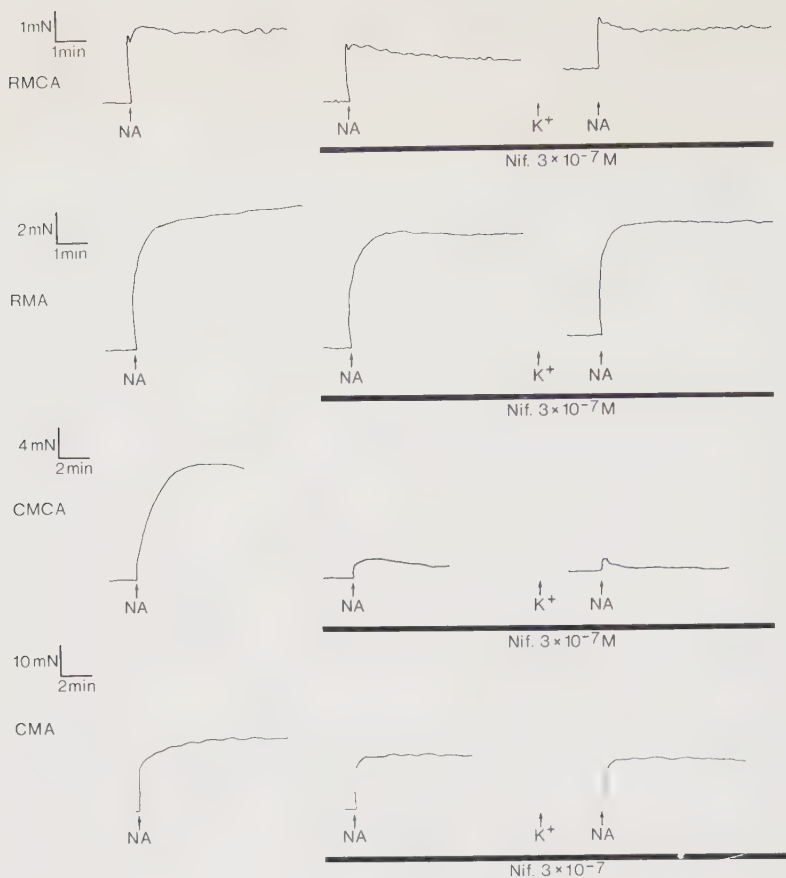


Fig. 8. Effect of prior K^+ depolarization (in the presence of 0.3 μ M nifedipine) on the contractile response to 10 μ M noradrenaline (NA) in rat and cat middle cerebral (RMCA, CMCA) and mesenteric (RMA, CMA) arteries. Results from four different experiments are shown. All vessel segments were treated identically. From the left to the right: **First arrow**, control contraction induced by NA in standard Krebs solution. **Second arrow**, response to NA in the presence of nifedipine (horizontal bars). Nifedipine (Nif.) was added 15 min before NA and then kept present throughout the experiment. **Third arrow**, exposure to a high K^+ solution, containing 124 mM K^+ . This evoked a transient contraction (not shown), which stabilized slightly above the baseline tension after 5 to 10 min. **Fourth arrow**, application of NA 5 to 10 min after introduction of K^+ .

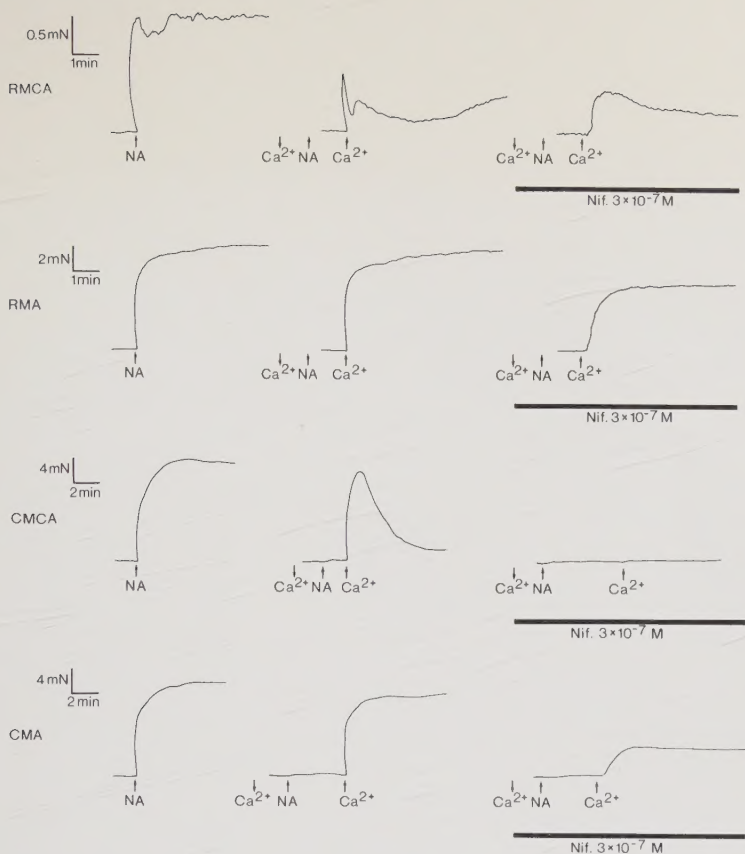


Fig. 9. Effect of nifedipine (Nif.) on Ca^{2+} -induced contractions in noradrenaline (10 μM)-exposed rat and cat middle cerebral (RMCA, CMCA) and mesenteric (RMA, CMA) arteries. The same experimental protocol was used in all experiments. From the left to the right: **First upward arrow**, control contraction elicited by noradrenaline (NA) in standard Krebs solution. **First downward arrow**, removal of Ca^{2+} by replacement with a Ca^{2+} -free Krebs solution (10 μM EGTA). **Second upward arrow**, introduction of NA to the bath solution. This sequence was repeated until the response to NA in Ca^{2+} -free medium was reduced to a minimum. **Third upward arrow**, readdition of 1.5 mM Ca^{2+} to the bath solution. The Ca^{2+} -induced contraction was subsequently repeated a second time in the presence of nifedipine (0.3 μM). The exposure time for nifedipine was 15 to 20 min.

